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of the
INSTITUTUM DIVI THOMAE

VOLUME II

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"Sicut medicus quamvis exterius operetur, natura sola interius operante, dicitur facere sanitatem; ita et homo dicitur docere veritatem quamvis exterius annuntiet, Deo interius docente."

Sancti Thomae Aquinatis
IX De Ver. Quaes. XI, Art. I, ad 7.

FOREWORD

The Institutum Divi Thomae is dedicated to fundamental research in the natural sciences. Its aim is the study of basic scientific problems in their broadest aspects. As a graduate school of research it will have affiliated units in universities, colleges, and hospitals who will cooperate in its research program. These units will look to the Institutum as a scientific center which will plan, direct and assign various phases of such basic research to its affiliated schools.

The first of these units was opened in September, 1938, at Rosary College, River Forest, Illinois. Sister M. Jordan, O.P., and Sister M. Veronita, O.P., who are the first graduates of the Institutum Divi Thomae, will carry on research at Rosary College in the fields of bacteriology and biochemistry. They will make regular reports to the Institutum and will have periodic conferences with the members of the Institutum faculty. Their findings will be published in this journal and·other scientific publications.

Definite arrangements are now being made for the establishment of three, and possibly four, other units during September, 1939.

EDITOR.

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OF THE
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GEORGE S. SPERTI, *Director*

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The Nihil Obstat and Imprimatur neither favor nor oppose opinions or theories here recorded. They are declarations that there is nothing in the Studies of the Institutum Divi Thomae contrary to faith or morals.

STUDIES

OF THE

INSTITUTUM DIVI THOMAE

SUPREME INTELLIGENCE AND SCIENCE

By REV. JAMES W. O'BRIEN* and GEORGE SPERI SPERTI

THE modern world has broken ties with the faith and authority of the past. It has seen the remarkable progress of science, and has been led to glorify reason beyond measure. Today everyone wants to be a rationalist. In the proper sense of the word that is exactly what everyone is. Man is by nature rational. Man is a rationalist when he works things out according to reason. And whether he arrives at truth through observation and experiment as does the scientist, or through rational faith in an infallible authority in matters of faith and morals, man is using reason. And yet some, who extol science beyond its just deserts, exclude from the province of rationalism everything that pertains to the world beyond the senses. Their rationalism is not rationalism at all; it is an irrational sensism. Nevertheless, they are satisfied with it, and like to call themselves rationalists in opposition to those who profess religious beliefs. They oppose especially Catholics, who insist on the importance of faith and authority more than on observation and scientific experiment for religious certitude. And because those who accept religious teaching admit that truth can also be found outside the field of science, they are scorned and mocked as the most gullible and deluded of men.

The use of the term rationalist to designate that class of people avowedly opposed to revealed religion leaves the impression that non-religion is founded upon reason, whereas religion is not; and in the minds of many a wide chasm has been opened up between religion and science. We are told that a person may be a believer in religion, or he may be a follower of science. He can not be both. A Catholic scientist is, in the view of these self-styled rationalists, as inadmissible as a scientific Catholic.

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The Catholic has always been called upon to defend the reasonableness of his faith. Nothing he says is taken for granted. On the other hand, anyone who wants to call himself a scientist is accepted without debate. It is assumed that all scientists, real and apparent, have reason on their side.

With the true scientist who recognizes the limitations of science and who realizes that there are any number of things that scientific experiment cannot attain and which science must, nevertheless, presuppose, the Church has no quarrel. She realizes the great benefits conferred upon mankind by the progress of science, and she knows she has nothing to fear from science, for truth is essentially one. As Pope Pius XI, recently addressing the priests of the world, said: The Church "blesses and fosters every healthy initiative and has no fear of the progress, even the most daring progress, of science, if only it be true science."[1] What is established as true and certain by the scientist must be accepted by the Christian as well. The same thing cannot be true in one field and false in another. It is the pseudo-scientist to whom the Catholic Church objects, partially because the pseudo-scientist delights in attacking her doctrine, but most of all because he is not a scientist. He is sailing under false colors. He is in reality a hypocrite. He accepts as true and certain what is, at most, probable or perhaps entirely unsupported by objective evidence, provided it is sufficiently materialistic in character.

The false scientist laughs at Catholics because they accept what to him are strange, impossible doctrines. If he would turn his critical eye to some of his own tenets, perhaps he would not be so derisive. Even deluded Catholics would find some of his scientific dogmas hard to believe. Ecclesiastical authority would find it difficult to make Catholics believe, for instance, that men are descended from monkeys, a doctrine which the pseudo-scientist accepts without hesitation. What answer will the pseudo-scientist give to this question: If it is perfectly rational to believe that men are the offspring of monkeys, what is irrational in believing that they are the children of one man and one woman?

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[1] Encycl. *Ad Catholici Sacerdotii,* 20 December, 1935.

The pseudo-scientist laughs to scorn transcendental, that is, suprasensual reality. His one criterion is observation and experiment. He refuses to accept anything that is beyond the world observed by the senses, and, though he is unaware of it, implicitly accepts many things that are not the objects of sense-perception. The complete evolutionary process which he proclaims has not yet been observed. Even if we were to assume that it can logically be deduced from effects which are observed, we would enter immediately into an argument which is by its nature transcendental, for the notions of cause and effect are outside the world of sense.

What scientist has observed the principle of contradiction or the capability of his intellect to attain truth? And yet, how fundamentally necessary are the principle of contradiction and the capability of the intellect to attain truth in order to have science at all? Of what use to prate about the descent of man from monkeys if at the same time it be admitted that his origin might as readily have been otherwise? Of what use to talk about the distances between planets and of their movements, of the interrelations of molecules, atoms, and electrons, if these things might just as well be not so. Why talk about objective reality if it may or may not be as we see it? How can the scientist speak about an atom, for instance, if his mind is not able to know with certainty that it is an atom? The scientist must admit some things that are beyond sense-perception or he falls into universal skepticism. If he admits that he is a universal skeptic, he is hardly a scientist.

The word scientist means one who knows something. A skeptic is one who does not. If, on the other hand, he accepts some things that are imperceptible by the senses, it is hard to see what is particularly rational about rejecting others.

The scientific non-religionists may be divided broadly into two classes, the Deists, on the one hand, and the Atheists and Agnostics on the other. In opposition to Theists, who believe in revealed religion, Deists reject the possibility or fact of revealed religion, although they admit upon philosophical and natural grounds a kind of first principle upon which all other things depend. Among the Deists are included such men as Voltaire, Rousseau, and Paine. It is difficult to see much difference between the theories of these men and those of absolute Atheists. It is not, however, the purpose of this paper to defend all the truths of revealed religion against their contentions. Its scope is limited to a refutation of the theories of the Atheists and Agnostics who deny that God exists, or maintain, at least, that it is impossible to know that He exists. Men falsely have been led to accept the conclusions of absolute atheism either upon pseudo-philosophical or pseudo-scientific grounds. In this category are included such men as Comte, Holbach, La Mettrie, Feuerbach, and Haeckel, who put forward systems that attempt to account for the universe without a self-existent intelligent God. Although, in this age of science, Atheism has become more and more common, there is nothing particularly scientific about it. Catholics have been so concerned about advancing arguments for the existence of God that they have forgotten to ask the Atheists for their proofs of His non-existence. No such proof has been advanced, unless ridicule and negative criticism be accepted as such. Their rationalism would resolve into absurdity if they were put on the defensive and asked to account by positive arguments for this remarkable universe without admitting an intelligent first principle.

This paper is directed equally against those known as Agnostics, who do not deny outright the existence of God but maintain that His existence cannot be demonstrated—a doctrine which is in no sense a modern one but is in accord with the views of Protagoras and Pyrrho. Its chief recent defender was Professor Huxley, who was accepted as a scientist.

If the ruin and confusion brought about by these so-called rationalists were confined to themselves, the situation would not be so bad. But such is not the case. Their influence has spread like a disease. Many persons unequipped intellectually to cope with these false views fall an easy prey to such pernicious doctrines, with results harmful to religion, to morality, and to society itself. This is especially true of young men and women who, while less capable of reasoned analysis than older people, are more exposed to the dangers of these baneful views.

Many young men and women go through a transitional period of doubt, during which they find their beliefs shaken to the very foundations by "advanced" reading and by the writings of the so-called "intelligentsia" who are unable to present an unbiased point of view, because of their inability to coordinate their higher learning with their religious beliefs. It seems that many young people, on reaching a certain stage in their quest for learning, become hopelessly swamped with atheistic ideas. They are neither farseeing nor persistent enough to hold on to their beliefs and to seek support for them instead of abandoning them. The true scientific point of view must be presented to such young people to help them to reestablish their religious beliefs on a more comprehensive basis.

If students of science studied and observed the almost unbelievably intricate design of nature, they would begin to perceive that this universe of ours, from the infinitesimal unit of matter, the atom, to the complicated galaxies and supergalaxies, is so well planned and so well organized that it could not possibly have been made by any force other than that of a Super-Intelligence. They would then finally be convinced of the existence of a Supreme Being or Divine Creator and would form very definite and sincere religious beliefs.

For their benefit we cite the doctrine of the Catholic Church, as proposed in the Vatican Council (1869-1870)[2] and in the oath against Modernism contained in the *motu proprio* "Sacrorum Antistitum" of Pius X,[3] September 1, 1910, which teaches that the existence of God can be known with certitude and can be demonstrated by the natural light of reason, which arrives at the conclusion of the existence of the Creator from the things that have been made. Thus, the Church makes her own the doctrine of St. Thomas and of practically all the theologians, concerning the natural means of knowing God. She speaks of scientific knowledge of the existence of God. The word "demonstrate" has a precise signification. It does not mean knowledge derived from feeling or confidence, nor even from faith, but from the purely intellectual process of induction, just as the conclusions of science are obtained. This is further confirmed by the use of the words "as cause (is known) through its effects." That is precisely the kind of knowledge science claims for itself. Furthermore, the argument does not lie entirely beyond the realm of sense-perception. There is a connecting link between the knowledge of the existence of God and the visible things of creation. The conclusions of science are no more evident. They, too, presuppose a relationship between intellectual knowledge and its object that transcends the power of the senses. The conclusions of science and the knowledge of God's existence presuppose a perception of material and sensible things, and both presuppose certain principles that lie beyond sensible things. The one, therefore, can be no more certain or evident than the other. If we accept the one, it is foolish to deny the other.

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[2] Denn. 1806: "If anyone should say that the one and true God, Our Creator and Our Lord, cannot certainly be known by the natural light of human reason, through the things that have been made, let him be anathema."

[3] Denn. 2145: "I confess that God, the beginning and end of all things, can be known with certitude and therefore also certainly demonstrated, by the natural light of reason, by means of the things that are made, that is to say, by the visible works of creation, as cause (is known) through its effects."

While the decrees just mentioned are comparatively recent, the doctrine itself is not new. The very same terms are found in the Epistle of St. Paul to the Romans: "For the wrath of God is revealed from heaven against all ungodliness and injustice of those men that detain the truth of God in injustice; because that which is known of God is manifest in them. For God hath manifested it unto them. For the invisible things of him, from the creation of the world, are clearly seen, being understood by the things that are made; his eternal power also, and divinity; so that they are inexcusable."[4]

From the context it is evident that St. Paul is speaking of the pagans and Gentiles, who were unacquainted with Divine Revelation. He is speaking, therefore, of a natural knowledge of God, obtained by the light of reason from the things that are made. When man knows the creature, therefore, according to St. Paul, he can know also the Creator.

The same doctrine of natural knowledge of God is contained also in the Book of Wisdom: "All men are vain in whom there is not the knowledge of God; and who by these good things that are seen, could not understand him that is, neither by attending to the works have acknowledged who was the workman; but have imagined either the fire, or the wind, or the swift air, or the circle of the stars, or the great water, or the sun and moon, to be the gods that rule the world. With whose beauty, if they, being delighted, took them to be gods; let them know how much the Lord of them is more beautiful than they; for the first author of beauty made all those things. Or if they admired their power and their effects, let them understand by them, that he that made them, is mightier than they. For by the greatness of the beauty, and of the creature, the creator of them may be seen, so as to be known thereby."[5]

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[4] St. Paul, I. Rom. i. 18-20.

This simple text is a clear and forceful refutation of the theories of those pseudo-naturalists who seek in nature alone the reasons for the existence and ordered motion of the universe. While this text contains the foundation upon which all five of the metaphysical arguments for the existence of God are built, it lays special emphasis upon those derived from the degrees of perfection and from the order in the universe. The greatness and power and beauty of the universe manifest how much greater is the power and beauty of God. "The heavens show forth the glory of God, and the firmament declareth the work of his hands."[6]

All of these texts speak of demonstration from effect to cause. The effect is the visible world about us. From a consideration of this visible world, which is physical in itself and and object of sense-perception, the mind naturally arrives at five abstract and metaphysical concepts. Upon these concepts the five metaphysical arguments for the existence of God are based.

> 1. The first argument is from motion. In the words of St. Thomas: "It is certain, and evident to our senses, that in the world some things are in motion."[7] Our experience, to be sure, attests that there is in the world not only motion from place to place, but also motion or change in the qualities of things such as is observed in material substances, in planets, in the atom. Experience attests also to spiritual motion or movements in the soul and in the soul's faculties of intellect and will. For motion, in its broadest acceptation, includes any change from potentiality to act, from

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[5] Book of Wisdom, xiii. 1-5.

[6] Psalm, xviii. 2.

[7] St. Thomas, *Summa Theologica*, I, q.2, a.3.

indetermination to determination. We have motion, therefore, when a body is moved locally, when the heat of a body increases, or when the soul through the intellect, for example, conceives an idea.

Having called attention to the fact that there are moving things which can be perceived by the senses, St. Thomas then goes on to prove the existence of God by means of two principles: first, that whatever is moved is set in motion by another; secondly, that in a series of actually and essentially subordinate movers, there cannot be an infinite succession of movers. We must finally arrive, therefore, at the conclusion that there exists a first mover, himself unmoved. It will be well to consider the very words of St. Thomas's argument taken from motion.

"Now whatever is in motion is put in motion by another, for nothing can be in motion except it is in potentiality to that towards which it is in motion; whereas a thing moves inasmuch as it is in act. For motion is nothing else than the reduction of something from potentiality to actuality. But nothing can be reduced from potentiality to actuality, except by something in a state of actuality. Thus that which is actually hot, as fire, makes wood, which is potentially hot, to be actually hot, and thereby moves and changes it. Now it is not possible that the same thing should be at once in actuality and potentiality in the same respect, but only in different respects. For what is actually hot cannot simultaneously be potentially hot; but it is simultaneously potentially cold. It is therefore impossible that in the same respect and in the same way a thing should be both mover and moved, i.e., that it should move itself. Therefore, whatever is in motion must be put in motion by another. If that by which it is put in motion be itself put in motion, then this also must needs be put in motion by another, and that by another again. But this cannot go on to infinity, because then there would be no first mover, and, consequently, no other mover; seeing that subsequent movers move only inasmuch as they are put in motion by the first mover; as the staff moves only because it is put in motion by the hand. Therefore it is necessary to arrive at a first mover, put in motion by no other; and this everyone understands to be God."[7]

2. The second argument is as follows: A succession of efficient causes, one acting upon another, can be perceived by the senses; causality cannot. It can be conceived only by the mind. Yet efficient causality is none the less real, and is constantly presupposed by scientists who contend that our knowledge is limited to sense-observation. If there is a dependent succession of causes, there must be one first independent efficient cause.

Experience shows that there is an order of efficient causes which is especially obvious in living things. Plants, animals, and men that are in the world today have not always been here. They have come into existence from their immediate forbears. Neither did their ancestors always exist; they in turn derived their existence from yet other ancestors. This fact is universally admitted, even by the evolutionists, who, however, assert that these ancestors need not have belonged to the same family. This ordered process of causes cannot go back indefinitely; there must have been at some time or other a beginning of that succession. It does not solve the problem to maintain, as do the evolutionists, that this succession had its beginning in some other species, for the problem immediately arises again with regard to that species. Logically, we must ultimately admit the existence of a first cause, uncaused. That first cause is God.

Again the words of the Angelic Doctor's second argument deserve serious study:

"In the world of sense we find there is an order of efficient causes. There is no case known (neither is it, indeed possible) in which a thing is found to be the efficient cause of itself; for so it would be prior to itself, which is impossible. Now in efficient causes it is not possible to go on to infinity, because in all efficient causes following in order, the first is the cause of the intermediate cause, and the intermediate is the cause of the ultimate cause, whether the intermediate cause be several, or one only. Now to take away the cause is to take away the effect. Therefore, if there be no first cause among efficient causes, there will be no ultimate, nor any intermediate cause. But if in efficient causes it is possible to go on to infinity, there will be no first efficient cause, neither will there be an ultimate effect, nor any intermediate efficient causes; all of which is plainly false. Therefore, it is necessary to admit a first efficient cause, to which everyone gives the name of God."[7]

3. The third argument of St. Thomas is taken from possibility and necessity. The fact that bodies come into being and cease to be, that is, that they are contingent, is apparent to the senses. Contingency, however, is not perceptible to the senses. Yet no one will deny that contingency has objective reality. From contingency we argue to one necessary being which must of its very nature exist.

For if creatures by their nature can be or not be, they have not within themselves the reason for their own existence. That existence must therefore be derived from something outside themselves. Again, unless an infinite process be admitted, we must admit the existence of a being that has within himself the complete reason for his existence. Such a being is not contingent but necessary. This necessary being is God.

The third argument stated in the words of St. Thomas runs thus:

"We find in nature things that are possible to be and not to be, since they are found to be generated, and to corrupt, and consequently, they are possible to be and not to be. But it is impossible for these always to exist, for that which is possible not to be at some time is not. Therefore, if everything is possible not to be, then at one time there could have been nothing in existence. Now if this were true. even now there would be nothing in existence, because that which does not exist only begins to exist by something already existing. Therefore, if at one time nothing was in existence, it would have been impossible for anything to have begun to exist; and thus even now nothing would be in existence—which is absurd. Therefore, not all beings are merely possible, but there must exist something the existence of which is necessary. But every necessary thing either has its necessity caused by another, or not. Now it is impossible to go on to infinity in necessary things which have their necessity caused by another, as has been already proved in regard to efficient causes. Therefore we cannot but postulate the existence of some being having of itself its own necessity, and not receiving it from another, but rather causing in others their necessity. This all men speak of as God."[7]

4. In the fourth place, from the varying grades of perfection in the universe we argue to a most perfect being, which is God. This is the argument that is chiefly inculcated in the quotation from the Book of Wisdom.

The fact that creatures differ in perfection shows that none of them are infinite or limitless, but that they participate in these perfections to a greater or less degree. It is impossible, therefore, that these creatures should have these perfections by their very nature. An increase or decrease in such a perfection, therefore, would mean an increase or decrease in the nature itself. It would cease to be this nature and would become something else. Since these perfections do not belong to creatures by their nature, they must be derived from some extrinsic cause. Again, unless we admit an infinite process we must logically arrive at a being which contains all these perfections, either formally or in an eminent degree. This being is God.

The fourth argument stated by St. Thomas is summed up in these words:

"Among beings there are some more and some less good, true, noble, and the like. But 'more' and 'less' are predicated of different things, according as they resemble in their different ways something which is the maximum, as a thing is said to be hotter according as it more nearly resembles that which is hottest; so that there is something which is truest, something best, something noblest, and, consequently, something which is uttermost being; for those things that are greatest in truth are greatest in being. Therefore there must also be something which is to all beings the cause of their being, goodness, and every other perfection; and this we call God."[7]

5. Lastly, there is the argument from order in the universe to a First Designer, an intelligent cause. This argument is peculiarly efficacious since it is based upon the mutual relations of efficient and final causes. It is an argument not only from efficiency but also from finality, or purpose, which presupposes intelligence. It is the argument with which we are chiefly concerned, since modern science has discovered an order both in the movements of the greatest of bodies, the planets, and the stars, and in those of the infinitesimal atom, undreamed of by the metaphysicians of the Schools or by the earlier philosophers.

The argument from order, as stated by St. Thomas, follows:

"The fifth way (of proving the existence of God) is taken from the government of things. We see that certain things which lack intelligence, i.e., natural bodies, operate on account of an end, which appears from this that always or usually they operated in the same manner in order to attain that which is best. Hence, it is obvious that they tend toward their purpose not by chance but by intention. Now those things that have not intelligence do not tend toward a purpose unless directed by some knowing and intelligent being, as an arrow by an archer. Hence, there is some intelligent being by which all natural things are ordered to a purpose and this being we call God."[7]

The argument may be summed up in the following conclusion: The admirable order in the world necessarily presupposes a most wise author, whom we call God.

All admit that in the world there is order, which is arrangement with a purpose. Order is the expression of design. The very word which was used by the Greeks is significant of this universal recognition of order. The word *cosmos* by which the Greeks signified the world, has for its primary meaning *order* as opposed to *chaos*. The progress of scientific investigation has increased our knowledge of the universe to a very great extent. No matter how minute or how large the body investigated, order reigns everywhere. This order is evident from all branches of science: Botany, Zoology, Chemistry, Physics, Astronomy, Physiology, Anatomy, Biophysics. It would be senseless for anyone to deny the fact. It would be even more foolish for the scientist to deny it, especially when consideration is given to recent scientific observations.

In developing the metaphysical arguments for the existence of God, two things must be kept distinct: first, the argument itself; secondly, the point from which the argument proceeds.

1. As regards the argument itself, it must be clearly understood that it does not give a "scientific demonstration" if the expression "scientific demonstration" is used in its modern acceptation, meaning a process that does not go beyond the data of observation and experience. It is, however, a "scientific demonstration" as Aristotle and the Scholastics understood this expression. According to Aristotle and the Scholastics an argument is not truly scientific unless it gives the "why" of what is affirmed. The natural or positive sciences, such as chemistry or physics, cannot give us this *raison d'être.* They give us the facts, but they cannot give us the reason why they are so and not otherwise. The only concern of the modern natural scientist is to classify the facts which he observes. Our reason, however, tells us that these facts are not self-explanatory and therefore must be explained by a cause which is beyond the limits of experience and observation. Only philosophy and metaphysics can attain this cause. The metaphysical arguments for the existence of God, therefore, though not "scientific" as moderns understand the expression, are, nevertheless, truly scientific.

2. The point from which the argument proceeds does lie within the fields of science, of experience, and of observation. That there is order in the universe is evident to all, but far more evident to scientists. Everyone can perceive the order in general, but the scientist can perceive the order of electrons in the atoms, of the elements in the universe, of the relations and movements of the planets. Hence, far

from detracting anything from the traditional metaphysical argument, modern science can only confirm it, as will be evident from a few examples of modern scientific discoveries.

To return to the first consideration regarding the metaphysical argument itself, we must observe that the empiricists deny that the principle of causality is a necessary truth, and that it permits us to go beyond the order of phenomena, to a necessary cause. This is the contention of Hume, Stuart Mill, William James, and others. Kant also maintained that it was impossible to prove the existence of God on the principle of causality.

The rejection of the principle of causality is at the basis of all objection to the arguments for the existence of God. The principle that there is nothing without a sufficient cause is one that is founded not upon experience, but upon ontological and transcendental reality. Natural science is unable to attain it, but still must presuppose it. It lies beyond the world of phenomena and hence is not limited to it. To reject this principle is to reject all reality and to fall into universal skepticism. For the sensible world with which science is so familiar is utterly bound up with this principle. Natural science presupposes it in supposing that there is such a thing as science, for there must be a causal relation between objective reality and man's own knowledge of that reality. This causal relation transcends the material object of science since it cannot be attained by experiment or observation. If we admit the principle of causality, and if there is order in the world (and science proclaims that there is), there must be an intelligent cause which disposes all things and guides them along definite lines to the achievement of the purpose of their existence. Even things so commonplace as the vitamins, when properly studied, offer evidence to substantiate the existence of a more than human designer.

Years ago it was found that substances of the "vitamin type" were essential for the prevention of deficiency diseases and for the maintenance of a condition of healthy resistance which sets up safeguards against susceptibility to infections in ways other than through the ordinary channels of immunology. Vitamins were found to be contained in natural food materials and to play an important part in the normal processes of nutrition. For example, cod-liver oil was discovered to be a rich source of vitamins A and D.[8] At first it was accepted only by quacks and, in general was regarded in much the same light as were the nostrums of those days. After the beginning of the present century, however, doctors began to realize the value of cod-liver oil and to administer it in prescribed doses.

Simultaneously with the discovery of the vitamins, it was found that sunlight and, in particular, ultraviolet light had the same beneficial effects as foods containing vitamins. The question of the interrelation of vitamins and sunlight led to the discovery that the formation of vitamin D is effected by sunlight.[9] It is an established fact that specific wave lengths of light promote the formation of vitamins while others destroy them. Radiant energy, on leaving the sun, contains not only the beneficial wave lengths but also the harmful ones. Were these wave lengths to reach the earth, according to the vast plan on which this system of light in relation to the vitamins is built, not only would the vitamins be destroyed but also many other essentials to life. One naturally asks the question: How does it happen that these harmful radiations do not find their way to the surface of the earth and destroy all life as we know it? We might guess that the oxygen, nitrogen, and other elements composing the earth's atmosphere, would remove or filter out the harmful wave lengths. Investigation, however, shows that these elements do not absorb the undesirable radiations to any marked degree. A more careful investigation reveals the fact that ozone has the quality we have been seeking, namely, that of selecting the correct wave lengths to an accuracy of a few hundred millionths of an inch.[10] What is the origin of this layer of ozone which is so helpful to mankind? A certain portion of the harmful radiations in the sun spectrum has the ability to convert the oxygen of the air into ozone.[10] The sun contains a certain band of harmful radiations which convert a portion of the oxygen of the air into a perfect filter for the removal of all undesirable radiations, but, at the same time, this filter has the property of transmitting the precise wave lengths which are essential to life on the earth. Are we to conclude that ozone just happened? Or, is the fact that it produces these beneficial effects an indication that is was destined for just that purpose? The minute accuracy and intricacy of this wave length selection is almost unbelievable. But when one realizes how disastrous would be the results if this specific wave length selection should shift in the slightest degree, it is apparent to the thinking man that this *cosmos* has a Director of more than human intelligence whom we know to be an Infinite Creator who has laid definite plans for our survival.

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[8] Osborne, T. B. and Mendel, L. B., *J. Biol. Chem.*, **17** (1914): 401; Clare, J. C. and Soames, K. M., *J. Am. Med. Assoc.*, **90** (1928): 770.

[9] Hess, A. F., *J. Am. Med. Assoc.*, **84** (1925): 1033, 1910.

In further support of this idea, the well-planned system might be mentioned by which carnivorous animals obtain their supply of fat-soluble vitamins. It would seem on the surface that animals, having for their food nothing but meats, might

[10] Ladenburg, R. W., *J. Optical Soc. Am.*, 25 (1935): 259.

lack these necessary elements. This is not true, since some land and water plants synthesize the vitamins A and D under the influence of sunlight and from them the animals of the land and sea derive a large percentage of their supplies. Carnivorous animals obtain them by preying upon other species which have stored the vitamins in a form which the meat-eating animals can assimilate. Ducks have a singular method of obtaining their supply of vitamin D. It is claimed that the duck's supply of this vitamin depends on the presence of a pro-vitamin stored in its preen glands which it is capable of bringing to the surface of its feathers, where it is converted by sunlight into vitamin D and taken into the duck's system in the process of preening its feathers.[11]

Eskimos, who are thoroughly shielded from sunlight and lack spinach and carrots and similar highly recommended vitamin-containing vegetables, by their survival are another argument for the order in the world. Diatoms, growing in the warmer waters, are very rich sources of pro-vitamin and when they are exposed to sunlight their pro-vitamins are changed to vitamins A and D. The small fish feed upon these diatoms and, by the process of "fish eat fish," the vitamins are finally stored in the fatty fractions and especially the liver oils of the fish inhabiting northern waters.[12] Eskimos, because of the cold climate, require foods of high fuel value. The fish fats available to them fill this need and it is through this channel that the Eskimos receive their vitamins. In brief, nature manufactures the vitamins at her best factory; preserves them properly within the fish; expresses them to the Eskimos who, although they do not know of their existence, need and want them. Must we suppose that all this is just an accident?

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[11] Hou, H. C., *Chinese J. Physiol*, 2 (1928): 345; 3 (1929): 171; 4 (1930): 79, 93, 345.

[12] Harris, L. J., Vitamins in Theory and Practice, Cambridge University Press, London, 1935; Sherman, H. C. and Smith, S. L., The Vitamins, Chemical Catalog Co., New York, 1931.

These and many other facts in the scientific field can be readily cited to verify the supremely important fact that we are living in a well-planned and regulated universe which the combined intelligence of mankind of all ages could not design and operate. It is no wonder that students, having worked in the scientific field, should be thoroughly convinced of the existence of a Supreme Intelligence or Almighty Creator.

A NOTE ON THE EFFECT OF TISSUE EXTRACTS UPON THE RESPIRATION OF YEAST*

BY SISTER M. VERONITA RUDDY, O.P.

THE presence of the bios group in normal animal tissues and tumors has been shown by Dittmar.[1] He found that extracts of kidney, adrenal body and liver, as well as extracts of other animal tissues produced stimulation of growth of yeast, the first mentioned producing the greatest stimulation. Experiments performed in our laboratories proved the possibility of stimulating growth, fermentation and respiration of yeast by means of a factor obtained from an irradiated yeast suspension,[2] substances isolated from malt combings[3] and some extracted from yeast.[4] It was the purpose of this research to determine whether the respiration of yeast can be influenced by tissue extracts and whether the effect is specific.

Extracts of kidney, spleen and liver of albino rats were tested for their effect upon the respiration of yeast cells. These extracts were prepared from 3 rats by pulping the tissues and mixing them with 10 times their weight of phosphate-glucose-Ringer solution. The suspensions were centrifuged, and the supernatant fluid poured off and autoclaved for 15 minutes at 20 pounds pressure. After autoclaving, the suspensions were again centrifuged and the supernatant fluids, which were now cell-free, were stored in sterile test tubes in the ice chest.

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*Acknowledgment is made to Dr. Robert J. Norris for his valuable suggestions.

[1] Dittmar, C., *Biochem. Z.*, 279 (1935): 99.

[2] Fardon, J. C., and Ruddy, Sr. M. Veronita, O.P., THESE STUDIES, 1 (1937): 41.

[3] Norris, R. J., and Kreke, C., THESE STUDIES, 1 (1937): 137.

[4] Norris, R. J., and Ruddy, Sr. M. Veronita, O.P., THESE STUDIES, 1 (1937): 53.

In determining the effect of these extracts upon the oxygen uptake of yeast, varying amounts of the extracts were placed in manometer flasks. Each flask also contained 1 cc. of a yeast suspension brought to a count of one* by means of the photodensitometer designed by Beck,[5] and sufficient phosphate-glucose-Ringer solution to bring the final volume to 3.1 cc.

In all the experiments the liver extract proved most potent. The increase over the control was 64 per cent with 0.1 cc., 225 per cent with 0.2 cc., and 357 per cent with 1.0 cc. of the extract. The first two tests had a respiration period of 1 hour, but for the last test the respiration period was only 20 minutes, after which the respiration measurements went beyond the limits of the instruments. Corresponding amounts of the kidney and the spleen extracts for the same periods of time gave the following results: kidney 20, 53 and 270 per cent; the spleen extract 8.7, 20 and 207 per cent.

The stimulation could not have been caused by the added respiration of any animal cells since these were completely absent from the extracts, nor by the oxygen consumption of the extracts themselves for this was found to be zero. Therefore the oxygen consumption was due to the yeast cells alone and this consumption was stimulated by the extracts.

CONCLUSIONS

In all cases there was a decided increase in the oxygen consumption of yeast cells.

The order of activity of the extracts was found to be liver, kidney, spleen.

The extracts were potent in minute quantities. When larger quantities were used the magnitude of the respiration was so great that it could only be determined for a short period of time.

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*Count 1 = 250,000 cells per cc.

[5] Beck, W. A., *Science*, 85 (1937): 368.

Original Manuscript submitted
June 15, 1938.

FRACTIONS FROM YEAST WHICH STIMULATE THE RESPIRATION OF YEAST AND ANIMAL TISSUES

By ELTON S. COOK, CORNELIUS W. KREKE AND
LEO G. NUTINI

The literature on bios[1] contains abundant references to the stimulation of growth and fermentation of yeast cells but few to respiratory stimulation. Copping,[2] however, has tested a bios preparation on yeast respiration with negative results.

It has been demonstrated in this laboratory[3] that cell-free fractions prepared from ultraviolet irradiated and non-irradiated yeast suspensions stimulate the respiration, growth and fermentation of living cells. A study of bios preparations (from uninjured cells) was undertaken with the object of detecting these multiple activities and, if possible, of separating and identifying the factors responsible for them. It has been shown[4] that the preparation of bios from yeast by Narayanan's[5] method gives fractions of varying respiration, growth and fermentation-stimulating activity on yeast; and maximum stimulation of any one type is exhibited by distinct fractions, suggesting very strongly the individual nature of the three factors. This conclusion seems to be supported by experiments on dialysis of these factors.[6] Furthermore, bios prepared from malt combings by the method of Lucas[7] shows that respiration, growth and fermentation-stimulating activities are resident to different degrees in the various fractions.[8] A preparation of bios from marmite, as employed by Copping,[2] also gave respiratory stimulation. The respiratory effect of sugar obliterated the stimulation of a crude bios made from cane sugar according to the method of Funk and Freedman.[9]

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[1] Tanner, F. W., *Chem. Rev.*, **1** (1925): 397.

[2] Copping, A. M., *Biochem, J.*, **23** (1929): 1050.

[3] Fardon, J. C., Carroll, Sr. M. Jordan, O.P., and Ruddy, Sr. M. Veronita, O.P., THESE STUDIES, **1** (1937): 17; Fardon, J. C., and Ruddy, Sr. M. Veronita, O.P., *ibid.*, **1** (1937): 41; Fardon, J. C., Norris, R. J., Loofbourow, J. R., and Ruddy, Sr. M. Veronita, O.P., *Nature*, **139** (1937): 589; Sperti, G. S., Loofbourow, J. R., and Dwyer, Sr. Cecelia Marie, S.C., *ibid.*, **140** (1937): 643; THESE STUDIES, **1** (1937): 163.

[4] Norris, R. J., and Ruddy, Sr. M. Veronita, O.P., THESE STUDIES, **1** (1937): 53.

[5] Narayanan, B. T., *Biochem. J.*, **24** (1930): 6.

[6] Norris, R. J., and Hart, Sr. M. Jane, O.P., THESE STUDIES, **1** (1937): 65.

In an attempt to determine the mechanism of the action of respiratory factors, we have shown[10] that preparations from both yeast and malt combings stimulate the respiration of yeast in the absence as well as in the presence of exogenous glucose but are inactive in the absence of the living cell. This we interpret as indicating that the factors directly stimulate cellular metabolic processes.

The question of specificity is one of great interest and has two aspects. First, the type of stimulation is probably specific, i.e., different metabolic processes are stimulated by different factors. This seems to be indicated definitely by the work quoted above. Second, the respiration of different tissues may be stimulated by different factors rather than by one general factor. It is with this second problem that we wish to deal in the present paper.

It has been found that extracts from liver stimulate the respiration of both liver and yeast as do extracts from malt combings.[11] However, a degree of specificity is indicated by the fact that liver extracts are more potent than malt combings extracts in stimulating liver respiration, and, similarly, malt combings extracts are more effective stimulants of yeast respiration than are liver extracts. That a greater degree of specificity is not found is probably due to the crudeness of the preparations since no attempt was made to fractionate them. Crude preparations from yeast and malt combings have also been found to stimulate the respiration of rat skin as well as of yeast.

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[7] Lucas, G. H. W., *J. Phys. Chem.*, 28 (1924): 1180.

[8] Norris, R. J., and Kreke, C. W., THESE STUDIES, 1 (1937): 137.

[9] Funk, C., and Freedman, L., *J. Biol. Chem.*, 56 (1923): 851.

[10] Cook, E. S., Hart, Sr. M. Jane, O.P., and Joly, R. S., *Proc. Soc. Exptl. Biol. Med.*, 38 (1938): 169.

[11] Ruddy, Sr. M. Veronita, O.P., *Arch. exptl. Zellforsch.* In publication.

The present paper represents the application of a simple fractionation procedure to yeast extracts in an attempt to secure fractions which might exhibit specificity in the respiratory stimulation of yeast and animal tissues. Although the method is not very efficient in a quantitative way it does indicate specificity and points the way for future studies. The chemical and absorption spectrum studies of the fractions, to be reported in a succeeding paper, should also suggest the course of future work.

EXPERIMENTAL

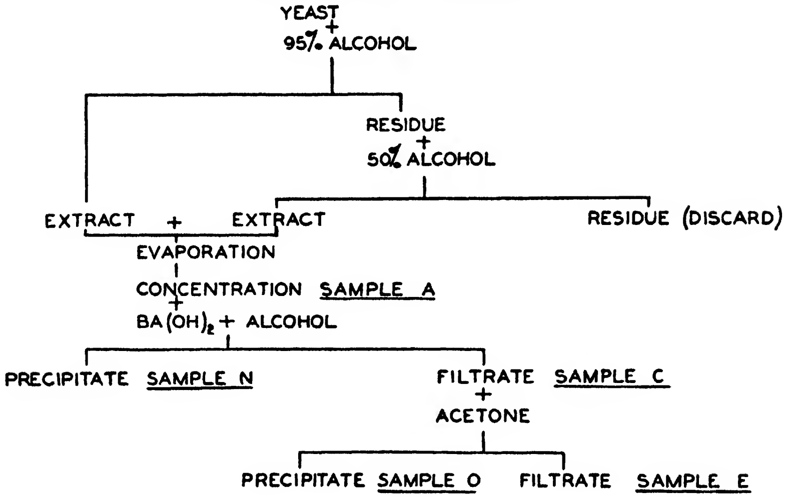
The source of the fractions was Fleischmann's bakers' yeast. The original extraction was based on Narayanan's[5] method while the fractionation was a modification of Lucas'[7] procedure.

Fractionation

Seven pounds of fresh Fleischmann's bakers' yeast were extracted with 2.5 liters of 95 per cent ethyl alcohol for 4 hours at a temperature of 60-70° C. with constant mechanical stirring. The alcohol lost during the extraction was continually replaced. This mixture was filtered through a Büchner funnel, the residue reextracted with 2.5 liters of 50 per cent ethyl alcohol under the same conditions, the extracts filtered until clear and the residues discarded.

The extracts were combined and evaporated to 400 cc. under reduced pressure at a temperature not greater than 60° C. A small part of this extract (25 cc.) was evaporated to dryness at 70° C. and labeled *Sample A*.

Key to Sample Number.


YEAST
+
95% ALCOHOL
RESIDUE
+
50% ALCOHOL
EXTRACT + EXTRACT
RESIDUE (DISCARD)
EVAPORATION
CONCENTRATION SAMPLE A
+
BA(OH)₂ + ALCOHOL
PRECIPITATE SAMPLE N
FILTRATE SAMPLE C
+
ACETONE
PRECIPITATE SAMPLE O
FILTRATE SAMPLE E

To 200 cc. of the extract were slowly added with constant stirring, 800 cc. of a hot saturated solution of barium hydroxide. A volume of 95 per cent ethyl alcohol, equal to twice the volume of the extract plus the barium hydroxide, was then added with constant stirring, which was continued for 10 minutes after the addition had been completed. The mixture was allowed to stand for 10 minutes longer and filtered. The filtrate was saturated with carbon dioxide by bubbling it through the liquid for 1 hour and then put in the ice chest until the treatment of the barium hydroxide precipitate had been completed.

The precipitate from the barium hydroxide-alcohol precipitation was stirred with a 100 cc. portion of distilled water at room temperature and filtered. After this operation had been repeated three times, the combined washings were saturated with carbon dioxide. The cloudy solution was reduced *in vacuo* at 40° C. to a volume of 54 cc. When the barium carbonate was filtered off and discarded there was left a clear reddish filtrate. This solution was heated to 60° C. and 2.0 cc. of 2.5 N sulfuric acid were slowly added with stirring. After the barium sulphate was allowed to settle and filtered off, the volume was 56.3 cc. Of this solution 16.3 cc. were evaporated to dryness at 70° C. and labeled *Sample N.*

The filtrate from the barium hydroxide-alcohol precipitation, which had been saturated with carbon dioxide, deposited on standing a precipitate of barium carbonate. The mixture, without filtration, was concentrated under reduced pressure to a volume of 150 cc. at 30-55° C. The precipitate was filtered off through a Büchner funnel. The filtrate was warmed to 60° C. on a water bath and 2.5 N sulfuric acid was added slowly with constant stirring until all the barium was precipitated; 22 cc. of 2.5 N sulfuric acid were required. The barium sulfate formed a colloidal suspension which was filtered out on an asbestos filter after standing overnight. The filtrate was perfectly clear and had a volume of about 175 cc. Of this amount 25 cc. were evaporated to dryness at 70° C. and labeled *Sample C.*

Three liters of acetone were added slowly, with shaking, to the remaining 150 cc. of the barium hydroxide-alcohol filtrate. The mixture was allowed to stand at room temperature for 72 hours. The acetone solution was then decanted from the red oily precipitate and the acetone was distilled off at room temperature until the clear solution became cloudy. The volume of the solution was 455 cc. of which 25 cc. were evaporated to dryness at 70° C. and labeled *Sample E.*

The red gummy residue from the acetone precipitation was washed with acetone and dried at 70° C. This was *Sample O.*

From the weights of the samples the total amount of solid in each fraction was calculated, and the results are shown in Table I. It is seen that 3.23 per cent of the original weight of yeast was contained in *Sample A* and was subsequently fractionated.

TABLE I

Solid Content of Samples

	Solid Content of Sample in g.	Total Volume of Each Filtrate in cc.	Solid Content of Total Fraction in g.
Sample A	6 4096	400.0	102 5536
" *N*	0.7003	56 3	2.4160
" *C*	5.9048	175 0	41.3336
" *O*	28.2757	.....	28 2757
" *E*	0.7035	455.0	12.8037

The dry solid of each sample was dissolved, just prior to biological assay, in distilled water to give a concentration of 50 mg. per cc. The pH of these solutions was adjusted to 7.3 with sodium hydroxide of such concentration that the solid content became 35-45 mg. per cc. Since these solutions are excellent culture media for bacteria, their respiratory stimulating activity was determined immediately after the solutions were prepared. It was later found that they could be sterilized at 20 pounds pressure for 20 minutes at a pH of 7.3 or 5.6 without loss of activity. Such solutions may be stored in stoppered flasks in the ice chest. Similar sterilization at a pH of 4 destroys the yeast activity and reduces the skin activity by half. Sterilization at a pH of 8 causes the pH to drop to 7 and reduces both the yeast and skin activity by 35-40 per cent.

Assay for Respiratory Stimulations

The direct manometric method of Warburg[12] was used for testing the respiratory stimulating activity of the samples on yeast and the two animal tissues, rat skin and liver.

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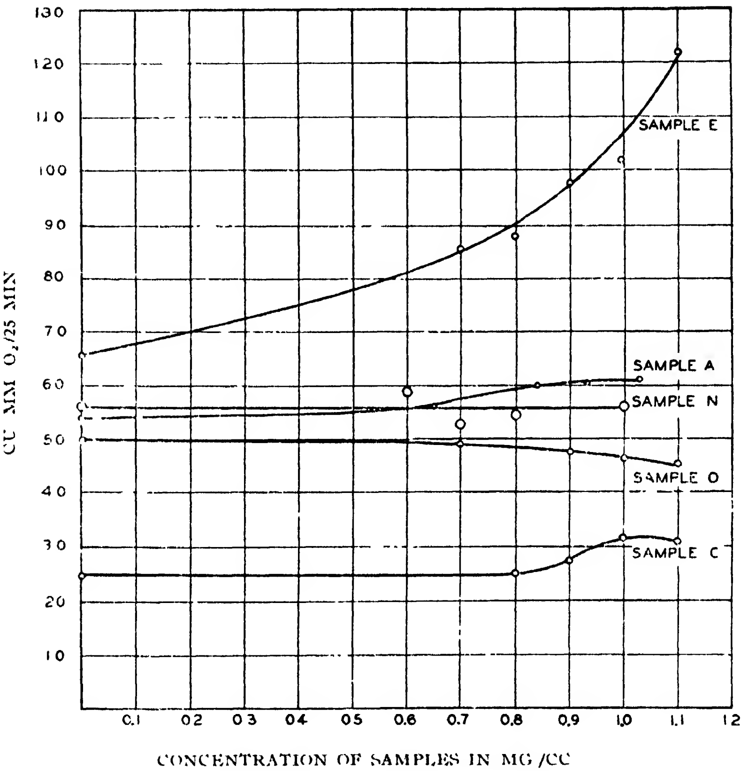
[12] Warburg, O., *Z. Physiol. Chem.*, **92** (1914): 231; *Biochem. Z.*, **142** (1923): 317; **152** (1924): 51; Warburg, O., and Kubowitz, F., *Biochem. Z.*, **214** (1929): 7; Warburg, O., Kubowitz, F., and Christian, W., *ibid.*, **242** (1931): 170.

FIG. 1—*Respiration Activity of Samples on Yeast*



Yeast Technique. This technique was the same as that previously described by Norris and Kreke.[8] The respiration period was 25 minutes, preceded by an equilibrium period of 15 minutes. The yeast count in the manometer flasks was 250.* The temperature of the water bath was 37.5° C.

The results of the experiments are shown graphically in Fig. 1. In Fig. 4 the effects of these samples upon yeast are compared with their effects upon rat skin and liver, where the

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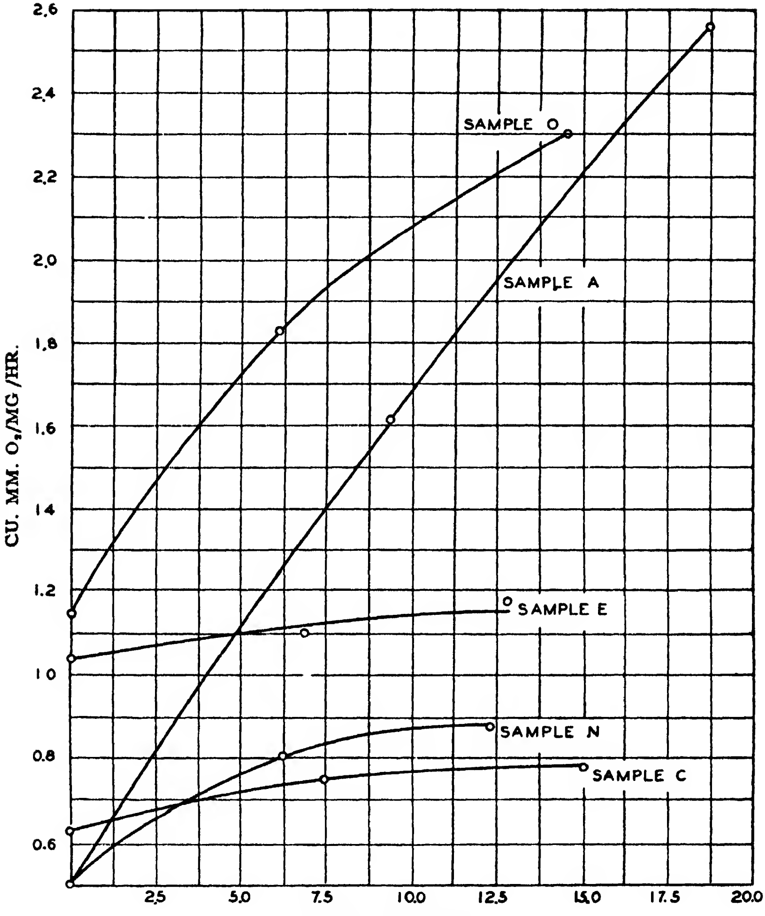
*Count 1 = 250,000 Cells per cc.

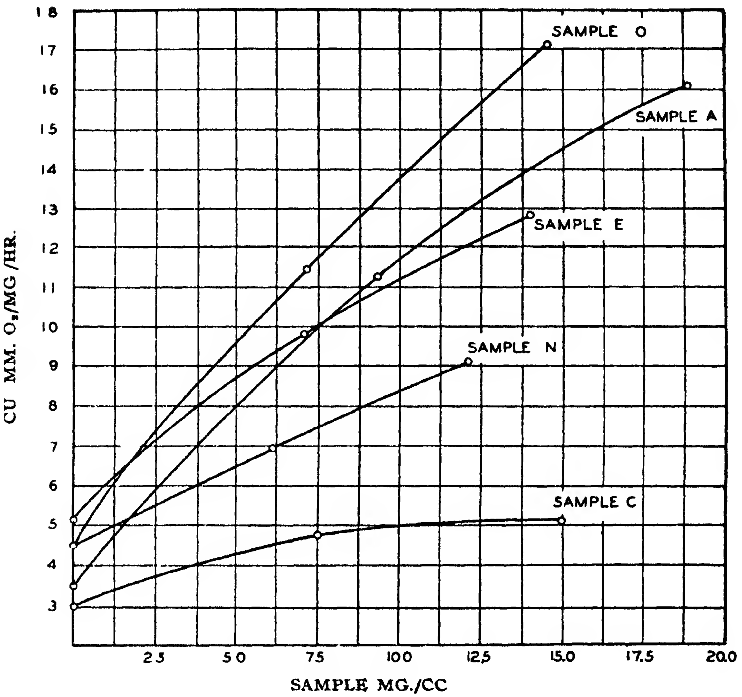
FIG. 2—*Respiration Activity of Samples on Skin*



yeast results are expressed as the per cent stimulation for 1 mg. of stimulating factor per cc.

Skin Technique. As in the yeast measurements, a bank of 7 manometers was used. These were divided as follows: one barometric control, two skin controls and four experimentals. All flasks contained 0.2 cc. of N KOH in the inner well and

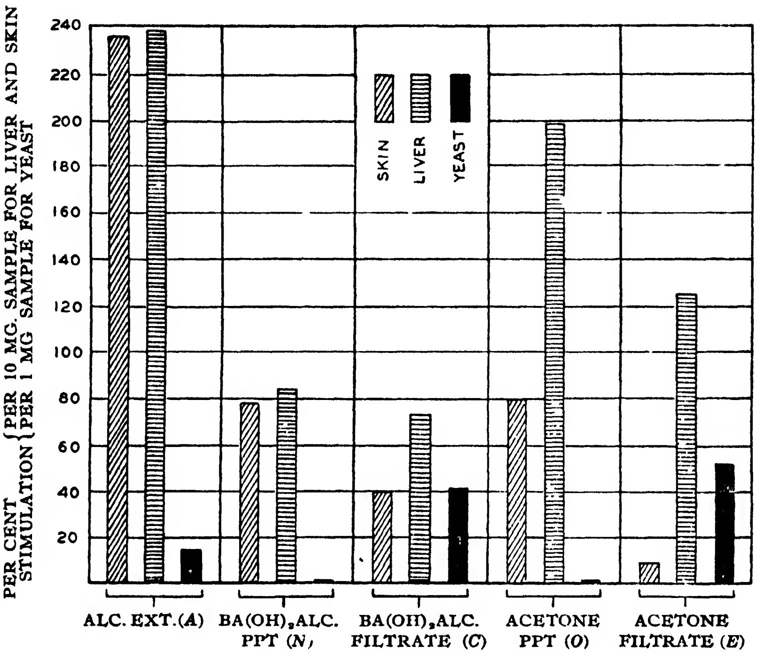
FIG. 3—*Respiration Activity of Samples on Liver*



the barometric and skin controls contained 3.1 cc. of Ringer-phosphate-glucose solution (pH=7.3) in the outer well. The test flasks contained, besides the samples, enough Ringer-phosphate-glucose to make the total volume in the outer well 3.1 cc. The temperature was 37.5° C.

The abdomen of a female albino rat about 1 year of age was shaved and the animal decapitated. Immediately after, a piece of skin 2 x 5 cm. was removed from the shaved area and placed in Ringer-phosphate-glucose solution, care being taken to remove all possible fat. The skin was cut into pieces 1 mm. square and each control and test flask was charged with 20-40 mg. (dry weight) of skin. The manometers were shaken for a

Fig. 4—*Potency of Fractions Stimulating Respiration of Liver, Skin and Yeast*



15-minute equilibrium period with the stopcocks open. After closing the stopcocks, the manometers were shaken for a 2-hour respiration period. A 2-hour rather than a 1-hour period was used to increase the total oxygen uptake and thus decrease the per cent of error. This seemed desirable on account of the relatively low respiration of skin. After taking the final reading the skin was removed from the flasks, washed with water, and dried overnight at 70° C.

The effects of the various samples on the respiration of skin are shown in Fig. 2. (The oxygen uptake is calculated and expressed in cubic millimeters per milligram of skin per hour.) It will be noted that the controls for *Sample O* and *Sample E* are almost twice as high as the controls for the others. This is due to the fact that male rats were used in these experiments. While insufficient experiments have been carried out to demonstrate beyond doubt a sexual difference in oxygen uptake of abdominal rat skin, our experience has been that the skin of male rats has an oxygen uptake of 1-1.15 cu.mm. per mg. per hr., whereas the corresponding values for females of the same age (one year) are 0.45-0.65 cu.mm. per mg. per hr. However, the percentage stimulation of a given fraction is very nearly the same irrespective of the sex of the rat. In Fig. 4 is shown the percentage stimulation given by 10 mg. of the samples per cc.

Liver Technique. The seven manometer flasks were charged as in the preceding experiment except that liver was substituted for skin. Ten to 20 mg. (dry weight) of liver slices, approximately 0.3 mm. in thickness, were placed in each flask. The liver was obtained from a female albino rat, one year old, and was placed in Ringer-phosphate-glucose solution at 37.5 C. One lobe of the liver was removed and thin sections were cut from it with a sectioner which was constantly wetted with Ringer-phosphate-glucose solution. The slices were immediately returned to the solution at 37.5° C., and no lobe was kept from the warm solution longer than 15 minutes. From 5 to 7 slices were placed in each manometer flask. The respiration period used in the liver experiments was 30 minutes. After the respiration measurements, the slices were washed, dried and weighed as described under *Skin Technique.*

The effects of the different fractions on the respiration of liver are shown in Fig. 3. The oxygen uptake is expressed in cubic millimeters per milligram of liver per hour. Fig. 4 shows the percentage stimulation given by 10 mg. of the samples per cc.

TABLE II

Comparison of the Potencies of Various Fractions in Stimulating the Oxygen Uptake of Yeast, Skin and Liver

	Total Number of Grams of Sample	Per Cent Stimulation of 1 mg./cc. on Yeast	Per Cent Stimulation of 10 mg./cc. on Skin	Per Cent Stimulation of 10 mg./cc. on Liver	Total Stimulation of Sample on Yeast	Total Stimulation of Sample on Skin	Total Stimulation of Sample on Liver
Sample A	102.5536	15	236	238	1,538	24,203	24,408
" *N*	2.4160	0	78	84	0	188	203
" *C*	41.3336	21	20	73	868	827	3,017
" *O*	28.2757	0	79	199	0	223,378	562,686
" *E*	12.8037	53	8.3	136	679	106	1,741

DISCUSSION

Figure 4 compares the potencies of the various fractions in stimulating the oxygen uptake of yeast, skin and liver which are expressed in percentage stimulation of 1 mg. per cc. of sample for yeast and 10 mg. per cc. for skin and liver. The stimulation refers to the following quantities of tissues and periods of respiration:

Tissue	Amount	Respiration Period
Yeast	Count of 250	25 min.
Skin	1 g.	120 min.
Liver	1 g.	30 min.

These same results are tabulated in Table II together with the total amount of stimulation of each sample, which was computed by multiplying the total weight of the sample by its percentage of stimulation. It should be recalled that the fractions labeled *Sample N*, *Sample C*, *Sample O* and *Sample E*, represent but half of the original extract, *Sample A*.

These results would seem to indicate clearly that the yeast active factor is distinct from the factor (or factors) active on animal tissues since two fractions were obtained which were devoid of yeast activity (*N* and *O*) but possessed tissue activity. Most fractions indicate a similarity between the skin and liver factors but a possible difference is suggested by the fact that *Sample E*, while possessing marked yeast and liver activity, is, within experimental error, almost devoid of skin activity. Indeed, earlier fractionations by this same method have given a *Sample E* without activity on skin but these earlier fractions were not tested on liver.

It is apparent that the fractionation process is not very efficient, particularly in regard to the skin factor. While the barium hydroxide precipitate is free of yeast activity, part of the skin activity goes into the filtrate. No fraction has the skin activity of the original extract. However, in experiments with crude extracts of low skin activity (40-50 per cent stimulation), the barium hydroxide-alcohol precipitation increases the skin activity of the precipitate approximately to the values shown here. One reason for this frequent loss of skin activity is found in the fact that the concentration of the crude extract is likely to cause the steam distillation of a small amount of a semicrystalline substance which, in the concentrations usually employed, stimulates respiration of yeast but not of skin. If this steam distillate be added in the proper proportion to the residual extract, a marked increase in the stimulation of skin respiration results. Thus, the problem of respiration stimulation is probably not simple. Investigation of the steam distillate is reported in a later paper from these laboratories.[13]

The fractionation is most efficient with the yeast factor, a progressive increase in potency being observed. This contrasts with the same fractionation process applied to malt combings where the yeast respiration-stimulating factor was found divided between the barium hydroxide precipitate and filtrate.[8]

The liver factor follows the skin factor in the barium hydroxide precipitation but is widely distributed in the fractionations that follow. There is not as much loss in potency in case of the liver factor as with the skin factor.

CONCLUSIONS

Yeast contains factors which stimulate the respiration of yeast, rat skin and rat liver. The yeast and animal tissue factors appear to be distinct, it being possible to prepare fractions having tissue activity but no activity on yeast. Further evidence for this is found in the difference in heat stability of the yeast and skin factors at a pH of 4. A possible difference between skin and liver factors is indicated since *Sample E* possesses yeast and liver activity, but little or no skin activity. The fractionation process described is least efficient in concentrating the skin factor. Reasons for this have been suggested. It is, however, efficient with the yeast factor.

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[13] Cook, E. S., and Kreke, C. W., THIS ISSUE, p. 47.

LITERATURE CITED

Cook, E. S., Hart, Sr. M. Jane, O.P., and Joly, R. S., *Proc. Soc. Exptl. Biol. Med.*, **38** (1938): 169.

Cook, E. S., and Kreke, C W., THIS ISSUE, p. 47.

Copping, A. M., *Biochem. J.*, **23** (1929): 1050.

Fardon, J. C., Carroll, Sr. M Jordan, O.P., and Ruddy, Sr. M. Veronita, O.P., THESE STUDIES, **1** (1937): 17.

Fardon, J. C., Norris, R. J., Loofbourow, J. R., and Ruddy, Sr. M. Veronita, O.P , *Nature*, **139** (1937): 589.

Fardon, J C , and Ruddy, Sr. M. Veronita, O.P., THESE STUDIES, **1** (1937): 41.

Funk, C., and Freedman, L., *J Biol Chem.*, **56** (1923): 851.

Lucas, G H. W., *J. Phys Chem.*, **28** (1924): 1180.

Narayanan, B T., *Biochem J.*, **24** (1930): 6

Norris, R J., and Hart, Sr. M. Jane, O.P., THESE STUDIES, **1** (1937): 65.

Norris, R. J., and Kreke, C. W., THESE STUDIES, **1** (1937): 137.

Norris, R. J., and Ruddy, Sr. M. Veronita, O.P., THESE STUDIES, **1** (1937): 53.

Ruddy, Sr. M. Veronita, O.P , *Arch. exptl. Zellforsch.* In publication.

Sperti, G. S., Loofbourow, J. R., and Dwyer, Sr. Cecelia Marie, S.C., *Nature*, **140** (1937): 643.

Sperti, G. S., Loofbourow, J. R., and Dwyer, Sr. Cecelia Marie, S.C., THESE STUDIES, **1** (1937): 163.

Tanner, F. W., *Chem. Rev.*, **1** (1925): 397.

Warburg, O., *Z. Physiol. Chem.*, **92** (1914): 231; *Biochem. Z.*, **142** (1923): 317; **152** (1924): 51.

Warburg, O., and Kubowitz, F., *Biochem. Z.*, **214** (1929): 7.

Warburg, O., Kubowitz, F., and Christian, W., *Biochem. Z.*, **242** (1931): 170.

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GROWTH-PROMOTING SUBSTANCES FROM INJURED TISSUE IN VITRO*

By JOHN C. FARDON and REV. WILLIAM A. SULLIVAN, O.P.

ALTHOUGH Lewis and Lewis[1] have shown that explanted fragments of embryonic tissue can be kept alive in a simple saline solution for periods ranging from a few hours to several days, with migration and some active cell division, Fischer[2] points out that these cells multiply at the expense of the nourishing and growth-promoting substances contained in the cells of the tissue explant. Burrows[3] has shown that by washing the tissue with a stream of serum, the stimulating substances from disintegrating cells are carried away and the degeneration of the central portion of the fragment is delayed, as are also the migration and multiplication of the cells. It is generally believed that the disintegration of some cells liberates growth-stimulating substances for other cells. Drew,[4] for instance, in studying the growth of adult tissues *in vitro* entertained the view that the products of autolysis from disintegrating cells become sufficiently concentrated after a lag period of some 10-13 days to initiate cell proliferation. Though apparently making no special issue of the observation, Walton[5] noted that if a piece of rapidly growing tissue *in vitro* was cut in two, growth was delayed on the cut edge, apparently from trauma, so that when the cells from the uncut edges had formed a halo of branching cells spreading out into the plasma, only a few cells were projecting from the cut portion. These cells, however, grew rapidly and after the second or third day nearly equalled in width the outgrowth from the uncut edges. In these laboratories we have frequently noted a similar phenomenon. A fragment of embryo liver was cut from the whole organ in such a fashion as to expose definite cut areas and an area forming the natural boundary of the organ. As shown in Plate I, the cut areas (A and B) formed the focus of migration and proliferation, while the smooth, uncut portions (C and D) revealed but slight outgrowth into the surrounding media. A phenomenon, related in principle to the above, has also been observed by the authors when tissues which showed no growth after a period of incubation were induced to grow after exposure to X-rays. A sufficient number of experiments, however, have not yet been conducted to warrant definite conclusions.

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*Research conducted under the Sir Charles F. Williams Fellowship.

[1] Lewis, M. R., and Lewis, W. H., *J. Am. Med. Assoc.*, 56 (1911): 1795.

[2] Fischer, A., Tissue Culture, Levin & Munksgaards, Copenhagen, 1917.

[3] Burrows, M. T., *Proc. Soc. Exptl. Biol. Med.*, 21 (1923): 94, 97, 102, 104.

[4] Drew, A. H., *Lancet*, 204 (1923): 785, 833.

[5] Walton, A. J., *J. Exptl. Med.*, 19 (1914): 123.

Many investigators have studied the mechanism of tissue repair in wound healing, especially in the field of plant physiology. Wiesner,[6] by producing injury to roots, leaf veins, beets, etc., found a congestion of plastic materials in the callus formation at the sectioned area.

Notable among the experimenters on animals is Carrel.[7] He found by actual experiment that a wound completely free from debris of tissue and blood clots and absolutely protected from outside irritation does not heal. He advances the opinion that the injury itself does not bring about the regenerative stimulus, but that consequent irritation initiates a mechanism which determines cell proliferation. The effect of every mild irritant, he goes on to say, is to bring about the invasion of leukocytes

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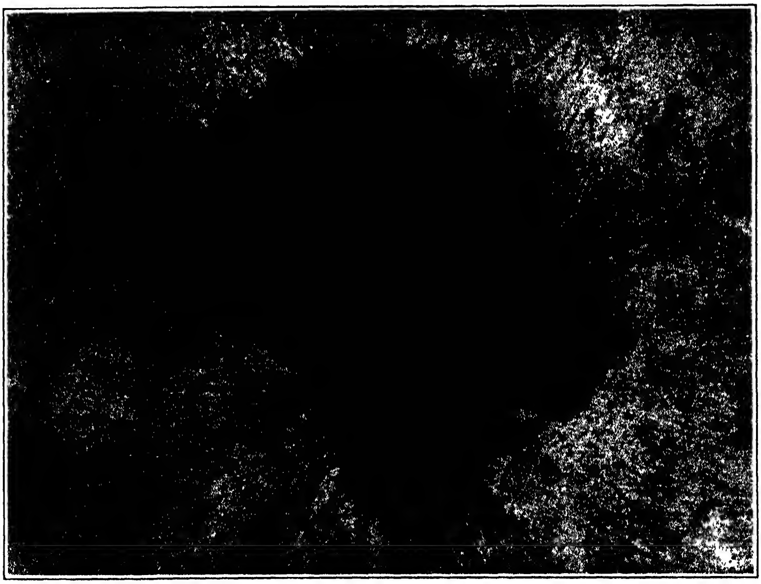
[6] Wiesner, J., Die Elementarstructur und das Wachstum der Lebenden Substanz, Vienna, 1892.

[7] Carrel, A., *J. Exptl. Med.*, 34 (1921): 425; *J. Am. Med. Assoc.*, 82 (1924): 255.

[8] Murphy, J. B., Liu, J. H., and Strum, E., *J. Exptl. Med.*, 35 (1922): 373.

PLATE I

Fragment of embryonic chick heart showing cut and uncut portions and comparative outgrowth.



The cut areas (A and B) formed the focus of migration and proliferation, while the smooth, uncut portions (C and D) reveal but slight outgrowth into the surrounding media

which in secreting "trephones" induce cicatrization. Murphy[8] found that after an animal has been exposed to X-rays, lymphocytes divide in its serum more readily than in that of an untreated animal. This effect Carrel attributes to the destruction or stimulation by X-rays of lymphocytes and the liberation of "trephones" in the plasma.

In these laboratories Fardon, Carroll and Ruddy,[9] as also Sperti, Loofbourow and Dwyer[10] have isolated metabolic stimulating factors from cells. In these experiments they have shown that injured cells secrete substances which stimulate growth, respiration and the utilization of sugars. Cognizant of the fact that injury (directly or indirectly) causes the secretion of growth-stimulating factors by certain cells *in vivo*, it occurred to us to investigate the importance of injury to tissues *in vitro*. The experiments described herein were conducted in an attempt to determine if growth-stimulating factors are present in tissues which have been freshly cut and which have not been permitted to remain in the media long enough to exhibit degeneration. It was further planned to ascertain whether these factors can be removed to a great extent by previously washing the tissue.

EXPERIMENTAL

Inasmuch as it is established that disintegrating cells supply a substance which stimulates the growth of tissues *in vitro*, and that in a saline medium degeneration of the outgrowth is known to commence in about three days, it was decided that the test period should not exceed two days. Although, in general, tissues exhibit new growth within 24 hours, fragments frequently fail to show growth until about the second day of

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[9] Fardon, J. C., Carroll, Sr. M. Jordan, O.P., and Ruddy, Sr. M. Veronita, O.P., THESE STUDIES, 1 (1937): 17.

[10] Sperti, G. S., Loofbourow, J. R., and Dwyer, Sr. Cecelia Marie, S.C., THESE STUDIES, 1 (1937): 163.

TABLE I

Relation of Degeneration to Age of Culture

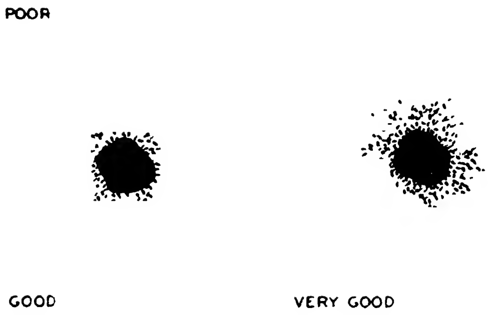
Slide No.	Seven-day Embryonic Chick Heart in Drew's Solution					
	17 Hours	24 Hours	41 Hours	65 Hours	96 Hours	140 Hours
1	Fair	No observation made	Good	Good D	Terminated	
2	Fair		Good	Good B		
3	Fair		Good	Good B		
4	Poor		Fair	Good		
5	Poor		Fair	Fair B		
6	Poor		Fair	Fair		
7	Fair		Fair	Fair		
8	Poor		Fair	Fair		
9	None		None	None		
10	Poor		Fair	Fair D		
11	Fair		Good	Good D		
12	None	Poor	Poor	No observation made	Poor D	Terminated
13	None	Poor	Fair		Fair D	
14	None	None	Poor		Fair	
15	Poor	Fair	Fair		Fair D	
16	Poor	Poor	Poor B		Poor D	
17	None	None	Poor		Poor D	
18	Poor	Poor	Fair		Fair D	
19	None	None	None		None	
20	None	None	Fair		Fair	
21	None	None	None		None	
22	None	None	None		None	
23	None	None	No observation made		No observation made	None
24	Good	V. Good				V. Good D
25	None	Poor				Poor D
26	Fair	Good B				Good D
27	Poor	Poor				Poor D
28	None	Fair				Fair D
29	None	None				None
30	Poor	Poor				Poor D
31	Poor	Poor				Poor D
32	None	None				Poor D
33	Poor	*				*
34	Poor	Fair				Fair D

*Contamination or drying of culture.

incubation. For this reason all observations were made upon the second day, allowing ample time for growth, but recording the data before sufficient waste products of metabolism could bring about degeneration of the outgrowth.

The preliminary experiment recorded in Table I bears out the above precautionary measures for observation of the

Fig. 1—*Chart of Growth Index*



cultures. It may be readily observed that many of the cultures in Table I which fail to show growth after 17 hours show definite growth after 41 hours. Readings taken after 65, 96 and 140 hours reveal the progress of ultimate degeneration. (D indicates complete degeneration; B, the beginning of degeneration.)

It might be well at this time to describe the method of culture preparation and the method employed for indexing the growth. All cultures were prepared according to the hanging-drop method of Lewis and Lewis.[1] Drew's saline solution was used without the addition of embryonic extract. Both embryonic heart and skin tissue of the chicken were used for explantation. The embryos were usually taken after the seventh or eighth day of incubation. The culture slides were placed in an incubator at 37 ° C. immediately after preparation.

TABLE II

The Effect of Washing on the Growth of Embryonic Chick Heart in Vitro

Slide No.	Unwashed 41 Hours	Washed 41 Hours	Slide No.	Unwashed 41 Hours	Washed 41 Hours	Slide No.	Unwashed 41 Hours	Washed 41 Hours
1	Fair	Poor	19	Fair	None	37	Fair	Poor
2	Good	Poor	20	V. Good	None	38	V. Good	Good
3	Good	None	21	Poor	Poor	39	V. Good	Good
4	Poor	Poor	22	Poor	None	40	Good	Poor
5	Poor	None	23	Fair	None	41	Good	Good
6	Poor	Poor	24	Fair	None	42	Good	Fair
7	Good	None	25	Fair	Poor	43	Fair	V. Good
8	V. Good	Poor	26	V. Good	Fair	44	Poor	Poor
9	V. Good	Good	27	Good	None	45	Good	Good
10	Good	Good	28	Fair	Poor	46	Fair	Good
11	Good	None	29	Good	None	47		Fair
12	Fair	Good	30	Poor	Poor	48		Good
13	V. Good	Fair	31	None	None	49		Good
14	Poor	Fair	32	Fair	Good	50		Poor
15	Good	Good	33	Fair	Good	51		None
16	None	Good	34	Good	V. Good	52		V. Good
17	Good	Fair	35	V. Good	V. Good	53		None
18	None	Poor	36	Good	V. Good			

UNWASHED

None.......... 3
Poor........... 8
Total..........11 = 24%

Fair.... 12
Good..... 15
V. Good..... 8
Total.........35 = 76%

WASHED

None..........14
Poor.........14
Total.........28 = 53%

Fair........... 6
Good.........14
V. Good...... 5
Total.........25 = 47%

All operations were conducted under aseptic conditions. Observations were made after 41 hours of incubation and recorded as: *no growth, poor, fair, good* and *very good* growth. The accompanying chart shows the relative amount of growth indicated by these terms. A total of 108 cultures were prepared for the experiment; some, however, were later discarded because of contamination.

The tissue for the controls was cut into small fragments and each piece immediately transferred to a slide. The fragments of tissue used in the experimental group were placed in a serological tube with Drew's solution and shaken gently for 30 seconds. This was repeated six times with every piece of tissue, the old fluid each time being pipetted off and replaced with fresh Drew's solution. Thereafter each fragment of tissue was transferred to a culture slide and incubated with the control group.

The results are recorded in Table II and may be summarized as follows: at the end of the 41-hour period only 24 per cent of the unwashed tissues showed growth ranging from *no growth* to *poor* growth, while in the washed tissues 53 per cent showed either *poor* or *no growth.* Contrariwise, only 47 per cent of the washed tissues showed growth ranging from *fair* to *very good,* while 76 per cent of the unwashed tissues exhibited growth ranging from *fair* to *very good.* The results clearly indicate that washing the tissues before explanting greatly reduces their growth rate *in vitro.*

SUMMARY OF RESULTS

1. To be significant, observations on embryonic tissue cultured in a saline fluid should be made after two days of incubation.

2. Cultures of unwashed embryonic tissue show greater outgrowth and migration of cells than tissues washed in several changes of saline solution before explantation.

CONCLUSIONS

It is apparent from the experimental data presented on tissue cultures that when explants are made, due consideration must be given to the growth-promoting factors which are liberated as a result of injury. Therefore, in determining absolute values of growth-stimulating substances on tissues *in vitro*, the tissues should be thoroughly washed in a saline fluid before explantation.

At the present time it cannot be definitely declared whether the growth promoters are secreted as a result of injury to the tissue, whether they are products from disintegrated cells or perhaps products liberated directly by living embryonic cells. However, in view of other experiments conducted in these laboratories we are inclined to believe that growth-promoting factors are released through direct tissue injury as well as from disintegrating cells.

LITERATURE CITED

Burrows, M. T., *Proc. Soc. Exptl. Biol. Med.*, **21** (1923): 94, 97, 102, 104.

Carrel, A., *J. Exptl. Med.*, **34** (1921): 425; *J. Am. Med. Assoc.*, **82** (1924): 255.

Drew, A. H. *Lancet*, **204** (1923): 785, 833.

Fardon, J. C., Carroll, Sr. M. Jordan, O.P., and Ruddy, Sr M. Veronita, O.P., These Studies, **1** (1937): 17.

Fischer, A., Tissue Culture, Levin & Munksgaards, Copenhagen, 1917.

Lewis, M. R., and Lewis, W. H., *J. Am. Med. Assoc.*, **56** (1911): 1795.

Murphy, J. B., Liu, J. H., and Strum, E., *J. Exptl. Med.*, **35** (1922): 373.

Sperti, G. S., Loofbourow, J. R., and Dwyer, Sr. Cecelia Marie, S.C., These Studies, **1** (1937): 163.

Walton, A. J., *J. Exptl. Med.*, **19** (1914): 123.

Wiesner, J., Die Elementarstructur und das Wachstum der Lebenden Substanz, Vienna (1892).

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A NOTE ON A SOLID FROM YEAST WHICH AFFECTS CELLULAR METABOLISM*

By ELTON S. COOK and CORNELIUS W. KREKE

In a previous paper on respiratory factors from yeast[1] it was mentioned that a small amount of a semicrystalline solid could be obtained during the concentration of the crude aqueous-alcoholic extract of yeast if the temperature were allowed to rise and permit, in effect, steam distillation. This substance is very interesting in its activity on several types of cellular metabolism. Preliminary experiments indicate that in the concentrations in which the other respiratory stimulating factors are active,[1] the steam distillate stimulates the respiration of yeast but depresses that of rat skin. In the usual concentrations[2] it appears to inhibit yeast fermentation and to be inactive or inhibitory on yeast growth; in the proper concentrations it stimulates proliferation in tissue cultures. The present note reports the method of preparation; its effect on the growth of tissues is discussed by colleagues in the next article.

EXPERIMENTAL

Seven pounds of Fleischmann's bakers' yeast were extracted with 95 per cent and 50 per cent alcohol.[1] If this extract is concentrated *in vacuo* at a temperature not greater than 60° C., no solid is observed in the distillate. On one occasion after most of the alcohol had been removed and the temperature allowed to rise above 70° C., a small amount of light yellow, semicrystalline solid was found in the distillate, which

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*Research conducted under the Sir Charles F. Williams Fellowship.

[1] Cook, E. S., Kreke, C. W., and Nutini, L. G., This Issue, p. 23.

[2] Norris, R. J., and Kreke, C. W., These Studies, 1 (1937): 137.

was acid to litmus. The solid was dissolved by adding normal KOH until the distillate was just basic. The solution was evaporated to a small volume over a Bunsen flame and taken to dryness on a water bath. A yield of 1.94 g. was obtained. This was 1.3 per cent of the total solids (148.90 g.) in the alcoholic yeast extract, or 0.06 per cent of the yeast.

In order to check the preparation of the solid by true steam distillation, the extraction of 7 pounds of yeast was repeated and the extracts were concentrated to 1100 cc. at 50° C. under reduced pressure. Next, 500 cc. of the concentrate were steam-distilled until all of the steam-volatile solid seemed to have been removed. About 2 liters of distillate were obtained. Sufficient 95 per cent alcohol was added to the distillate to bring the total volume to 3 liters. This dissolved the solid and gave a clear solution, 70 cc. of which required 1.4 cc. of 0.0714 N KOH to bring it to the phenolphthalein end-point. Since the solution was found to contain 0.42 mg. of solid per cc., this titration value would correspond to a neutralization equivalent of 295 for the solid. It should be noted that the neutralized solution, on standing for a time, was capable of taking up more KOH. Therefore the correct equivalent is somewhat smaller. To the remaining 2930 cc. of the solution, 58.6 cc. of 0.0714 N KOH were added and the solution concentrated *in vacuo* to a small volume at 30-57° C. The residual solution was taken to dryness at 70° C. in the oven. The yield (based on 1100 cc. of concentrate) was 1.2652 g., or 0.72 per cent of the total solids (173.85 g.). This corresponds to 0.04 per cent of the yeast. Although the yield was smaller than in the original run, the material thus obtained seemed considerably more potent.

The steam distillate comprises 0.04-0.06 per cent of the yeast and is a light yellow, waxy, semicrystalline solid, nearly insoluble in water but soluble in 95 or 50 per cent alcohol, ether, benzene or alkali. The iodine number is 5.5 by the Hanus method. The Liebermann-Burchard test for sterols is negative. On treatment with KOH solution the steam distillate forms a white or slightly colored, water-soluble, hygroscopic solid. This compound appears to have the properties of a salt since it does not melt at 360° C., although it begins to color at about 210° C. These properties suggest that the steam distillate consists largely of saturated fatty acids.[3]

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[3] Cook, E. S., and Kreke, C. W., *Nature*, **142** (1938): 719.

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THE EFFECT OF A STEAM DISTILLATE OF YEAST ON THE GROWTH OF EMBRYONIC TISSUE IN VITRO*

BY JOHN C. FARDON AND REV. WILLIAM A. SULLIVAN, O.P.

THE existence of growth-promoting substances has been suggested many times by various investigators. Carrel[1] has shown that extracts of various tissues, such as embryos, adult spleen, leucocytes and Rous chicken sarcoma, considerably increased the rate of growth of fibrocytes *in vitro.* Akamatsu[2] showed that after wounding, the plasma from an animal possessed more growth-promoting substances than before the wound was made. Drew[3] states that autolyzed normal adult tissue contains growth-stimulating substances which he claims have the power to cause adult tissues to proliferate in culture.

We have shown in these laboratories[4] that growth-stimulating substances are produced when cells are injured or destroyed. Although we have succeeded in isolating factors from cells which stimulate growth, the experiments so far reported have dealt primarily with relatively impure materials. Our colleagues, Cook and Kreke,[5] have reported a fat-soluble fraction produced from yeast (*Saccharomyces cerevisiae*) which has certain metabolic-stimulating powers. In view of our earlier experiments it was decided to test this metabolic-stimulating fraction (a steam distillate) for its ability to stimulate the growth of embryo tissue *in vitro.* Since our colleagues have already described the properties of this fat-soluble fraction, this paper will be confined exclusively to the biological investigation of the material.

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*Research conducted under the Sir Charles F. Williams Fellowship.

[1] Carrel, A., *J. Exptl. Med.*, **15** (1912): 393.

[2] Akamatsu, N., *Virchow's Arch. Path. Anat.*, **240** (1923): 308.

[3] Drew, A. H., *Brit. J. Exptl. Path.*, **4** (1923): 46.

[4] Fardon, J. C., and Sullivan, W. A., THIS ISSUE, p. 39.

[5] Cook, E. S., and Kreke, C. W., THIS ISSUE, p. 47.

EXPERIMENTAL

In the preparation of the cultures, the hanging-drop method of Lewis and Lewis[6] was adopted. The age of the embryo tissue was to some extent dependent upon the fertile chicken eggs available at the time of culture preparation. As a rule skin was taken from an embryo 5 to 8 days old. The pH of the Drew's saline solution was never permitted to vary beyond the limits of 6.8 to 7.0. This variation in pH, as determined by a series of separate experiments, makes little difference in the amount of growth produced. In no instance was embryonic extract added to the cultures, nor were subcultures prepared. The cultures were immediately placed in an incubator at 37.5° C., and as a rule the first observation was made after the second day. The method of recording the growth was the same as that employed in another investigation described in this issue.[4] The relative amount of growth in the cultures was denoted as: *none, poor, fair, good* and *very good.* Although this method of recording obviously has its limitations, simultaneous observations made by several observers checked to a degree of accuracy which made the method justifiable.

In the experimental group the steam distillate of yeast was added to the Drew's solution and thoroughly mixed. The sample (A_3) used in Experiments 1 to 5 was a water solution of the crystalline solid containing 27.7 mg./cc. with a pH of 7.3. Three different concentrations of this distillate were used, namely, 1 part of the distillate to 1, 3, and 6 parts of Drew's solution respectively. Experiments 6 and 7 were prepared with a sample (A_5) containing 47 mg./cc. Two concentrations of this sample, 1 to 16 and 1 to 6, were used. The control series always contained Drew's solution only. A total of 7 separate experiments were conducted, using altogether 164 cultures.

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[6] Lewis, W. H., and Lewis, M. R., General Cytology, University of Chicago Press, 1924, p. 385.

The results of the experiments are recorded in Tables I and II, wherein the distillate concentration, the number of slides per experiment, the kind and age of embryonic tissue as well as the growth rates are noted. Culture slides which became contaminated, loose, or dry, or were stained for photomicrographic purposes have been indicated.

The variations in growth were typical of those found in tissue culture experiments. In the first series of experiments, the A_3 preparation was tested. This preparation was described by Cook and Kreke as an accidental product collected during a steam distillation. The preparation A_5 was the result of a direct attempt to steam distill the growth-promoting fraction. Cook and Kreke found that the A_5 sample was considerably more potent than the A_3 sample in stimulating the respiration of yeast. There was no reason to believe, however, that the respiratory-stimulating power of this sample was in any way related to its power to stimulate growth. The only method by means of which the growth-stimulating potencies of the A_3 and the A_5 samples could be compared was to test them for their power to promote the growth of tissues *in vitro*.

In Experiment 1, in which the A_3 sample was used, it may be noted that in the control series 58 per cent showed *none*, 33 per cent showed *poor* growth, and 9 per cent showed *fair* growth; that is, 91 per cent showed growth ranging from *none* to *poor* and 9 per cent showed *fair* growth. In the experimental series, 4 of the 12 cultures had to be discarded either because of contamination or excessive evaporation of the culture media, leaving only 8 for the experiment. Of these 8 cultures, 25 per cent showed growth ranging from *none* to

TABLE I

Effect of A_3 Distillate on Tissue Growth

Experiment 1 Age of Tissue, 5 days. Time of Observation, 41 hours. Distillate/Drew's, 1/3				Experiment 2 Age of Tissue, 6 days. Time of Observation, 41 hours. Distillate/Drew's, 1/3				Experiment 3 Age of Tissue, 7 days. Time of Observation, 65 hours. Distillate/Drew's, 1/3			
Slide No.	Growth of Control	Slide No.	Growth of Experimental	Slide No.	Growth of Control	Slide No.	Growth of Experimental	Slide No.	Growth of Control	Slide No.	Growth of Experimental
1	None	13	None	25	None	37	Fair	49	None	61	Good
2	None	14	*	26	Poor	38	Poor	50	*	62	Good
3	Poor	15	Good	27	None	39	Very Good	51	Fair	63	Good
4	None	16	Very Good	28	Poor	40	Fair	52	None	64	Poor
5	None	17	Good	29	Fair	41	Poor	53	None	65	Fair
6	Poor	18	Fair	30	Poor	42	Good	54	Good	66	Very Good
7	None	19	Fair	31	Poor	43	None	55	Good	67	Fair
8	Poor	20	Fair	32	None	44	Good	56	*	68	None
9	None	21	*	33	None	45	Good	57	Poor	69	None
10	Fair	22	Poor	34	Poor	46	None	58	None	70	Fair
11	Poor	23	*	35	Poor	47	Good	59	Fair	71	None
12	None	24	*	36	None	48	Very Good	60	Fair	72	None

*Discarded.

TABLE I (CONTINUED)

Effect of A_3 Distillate on Tissue Culture

EXPERIMENT 4 Age of Tissue, 6 days. Time of Observation, 41 hours. Distillate/Drew's, 1/6				EXPERIMENT 5 Age of Tissue, 9 days. Time of Observation, 39 hours. Distillate/Drew's, 1/1			
Slide No.	Growth of Control	Slide No.	Growth of Experimental	Slide No.	Growth of Control	Slide No.	Growth of Experimental
73	None	85	*	97	Good	109	Poor
74	*	86	Poor	98	Very Good	110	None
75	Good	87	*	99	Very Good	111	None
76	Good	88	Good	100	Very Good	112	Poor
77	Poor	89	Very Good	101	Very Good	113	*
78	None	90	Very Good	102	Fair	114	None
79	Poor	91	Very Good	103	Very Good	115	None
80	None	92	Fair	104	Good	116	None
81	Poor	93	Poor	105	Very Good	117	Poor
82	None	94	Poor	106	Fair	118	Poor
83	Poor	95	Very Good	107	Good	119	None
84	Poor	96	Fair	108	Good	120	None

*Discarded.

poor while 75 per cent showed growth ranging from *fair* to *very good.* It should be recalled that only 9 per cent of the control cultures showed *good* growth. It may be seen from this experiment that 1 part of sample A_3 in 3 of Drew's solution seems to have stimulated markedly the growth of 5-day embryo skin.

In Experiment 2 the A_3 distillate was again used with 3 parts of Drew's solution. In this experiment, 11 slides of the control series showed growth ranging from *none* to *poor*, while 1 showed *fair* growth. These results were the same as those of the control series of the previous experiment, namely, 91 per cent *none* to *poor* growth and 9 per cent *fair* growth. In the experimental series 33 per cent showed *none* to *poor* growth while 67 per cent showed growth ranging from *fair* to *very good.* The results of this experiment are substantially in agreement with those of the previous experiment, showing again that the A_3 sample of the steam distillate markedly stimulated the growth of embryo skin.

In the third experiment, 7-day-old skin was used. The experimental procedure was identical with that of the previous experiments except that the observations were made after a period of 65 hours. In this experiment, 2 of the cultures in the control series were contaminated. Of the remaining 10 cultures, 50 per cent showed *none* to *poor* growth and 50 per cent showed *fair* to *good* growth. In the experimental series, 42 per cent showed *none* to *poor* growth while 58 per cent showed growth ranging from *fair* to *very good.* It is seen in this experiment that the 65-hour test is not as satisfactory for testing growth promotion as the 41-hour test. With the longer test period degeneration products from dying cells probably supply a factor which stimulates the growth of the remaining living cells as suggested by Drew.[3]

In the fourth experiment 1 part of the A_3 sample was added to 6 parts of Drew's solution. In this series of tests, 82 per cent of the control series showed *none* to *poor* growth. Two of the controls (18 per cent) showed *good* growth. In the experimental group 30 per cent showed *poor* growth while 70 per cent showed growth ranging from *fair* to *very good.* From this experiment it may be seen that the 1 to 6 dilution of the A_3 sample showed approximately the same growth-stimulating power as the 1 to 3 dilution.

In Experiment 5, 1 part of A_3 was diluted with 1 part of Drew's solution. In this experiment in which skin from a 9-day-old embryo was used, the control series showed excellent growth. The excellent growth rate found in the controls is in agreement with repeated observations which indicate that 9-day-old embryo skin grows better than 5 and 6-day-old embryo skin. In the experimental series all the tissues showed growth ranging from *none* to *poor.* From this it may be clearly observed that too high a concentration of the growth factor is toxic for the cells and not only does not stimulate the growth but actually retards it.

The results of all the experiments with sample A_3 are given in Table I.

In Experiment 6, sample A_5 was tested. In this experiment the skin of a 9-day-old embryo was used resulting in *good* growth in the control series, while the tissues to which 1 part in 6 of the A_5 distillate was used showed no growth whatsoever. It will be recalled that in the previous experiment 1 part of A_3 in 6 parts of Drew's solution showed excellent growth. From this experiment it was therefore concluded that, due to the toxicity sample A_5 had shown when diluted to 1 part in 6 of Drew's solution, in all probability it was much more potent than sample A_3. As a result of this observation Experiment 7 was conducted in which 1 part of A_5 was added to 16 parts of Drew's solution. The results of this experiment were as follows: In the control series 75 per cent showed *none* to *poor* growth and 25 per cent *good* growth, while in the

TABLE II

Effect of A_5 Distillate on Tissue Growth

Experiment 6 Age of Tissue, 9 days. Time of Observation, 17 hours. Distillate/Drew's, 1/6				Experiment 7 Age of Tissue, 10 days. Time of Observation, 41 hours. Distillate/Drew's, 1/16			
Slide No.	Growth of Control	Slide No.	Growth of Experimental	Slide No.	Growth of Control	Slide No.	Growth of Experimental
121	Very Good	131	None	141	Poor	153	Fair
122	Fair	132	None	142	Poor	154	Good
123	None	133	None	143	Poor	155	Good
124	None	134	None	144	None	156	Very Good
125	Good	135	None	145	Good	157	Good
126	Good	136	None	146	Poor	158	Very Good
127	Very Good	137	None	147	Good	159	Fair
128	Very Good	138	None	148	Poor	160	Poor
129	Very Good	139	None	149	Poor	161	Good
130	Good	140	None	150	None	162	None
				151	None	163	Good
				152	Good	164	Good

TABLE III

Comparison of the Stimulating Effect of the Various Concentrations of the Steam Distillate

Distillate	Experiment Number	Tissue Age in Days	Distillate/Drew's Solution	Per cent *None* to *Poor* Growth		Per cent *Fair* to *Very Good*	
				Control	Experimental	Control	Experimental
A_3	1	5	1/3	91	25	9	75
A_3	2	6	1/3	91	33	9	67
A_3	3	7	1/3	50	42	50	58
A_3	4	6	1/6	82	30	18	70
A_3	5	9	1/1	0	100	100	0
A_5	6	9	1/6	20	100	80	0
A_5	7	10	1/16	75	17	25	83

experimental series 83 per cent showed growth ranging from *fair* to *very good* and but 17 per cent *none* to *poor* growth.

The results of all the experiments with sample A_5 are given in Table II.

In Table III is given a summary of the results of each experiment, that is, a comparison of the percentages of cultures recorded as *none* and *poor* growth, and the cultures recorded as *fair*, *good*, and *very good* growth.

A typical culture showing normal, healthy growth in a test is reproduced in Plate I. In the field can be seen fibrocytes protruding from the edges of the tissue overlaid with a pavement of epithelial cells which have grown out from the original explant.

A typical culture showing degeneration in a control is also shown in Plate I. This culture shows less outgrowth of both epithelial cells and fibrocytes and earlier indications of degeneration than the cultures to which the growth-promoting factors were added.

SUMMARY

1. A steam distillate prepared from yeast (*Saccharomyces cerevisiae*) having the property of inhibiting skin respiration at high concentrations and of remaining inactive or but slightly stimulatory at very low concentrations, has been found to stimulate the growth of embryonic skin *in vitro*.

2. The concentration of the yeast distillate in the cultures is an important factor. The same factor which in low concentrations stimulates growth, in higher concentrations has been found to greatly retard and in many cases to inhibit growth.

CONCLUSIONS

It appears from the foregoing experimental results that a fraction obtained from the steam distillation of yeast, which

PLATE I— EXPERIMENTAL

Two-day experimental cultures with steam distillate of yeast (A_3) showing growth of epithelial cells.

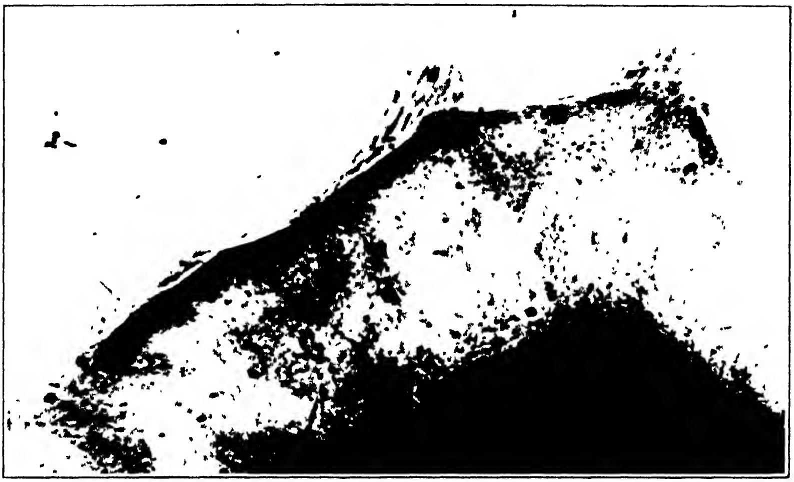
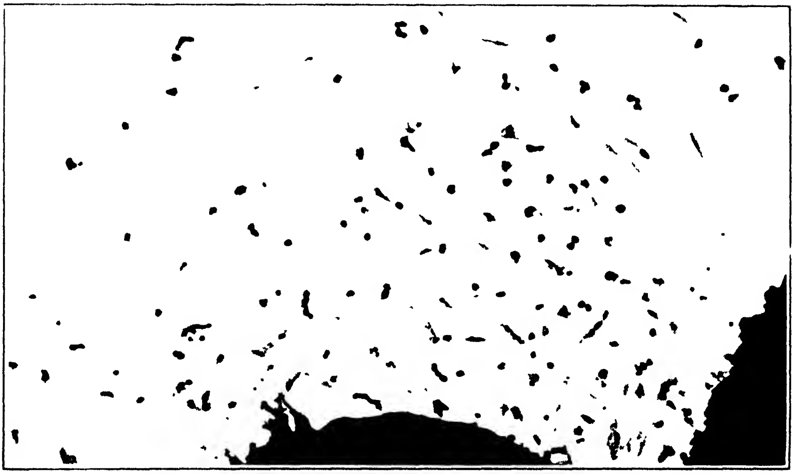


PLATE I— CONTROL

Two-day control culture with Drew's solution only, showing degeneration of outgrowth.



greatly affects such metabolic activities as respiration and fermentation in yeast cells and respiration of animal tissues, also contains a factor or has the concurrent ability to stimulate the growth of embryonic animal tissue *in vitro.*

The fact that in higher concentrations the distillate depresses growth may indicate that it acts as a mild irritant or injuring agent at lower concentrations causing the cells to secrete the growth factors. Such is the case found by Loofbourow *et al.*[7] of these laboratories concerning the action of heteroauxin.

Since Carrel[8] has shown that embryonic juice added in increased concentrations does not retard the growth of tissues *in vitro*, it seems highly improbable that the growth factor used in these experiments filled solely the role of a nutrient material. It is our opinion that the steam distillate might be considered a growth-stimulating agent of a hormone-like nature.

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[7] Loofbourow, J. R., and Dwyer, Sr. Cecelia Marie, S.C. *Science,* **88** (1938): 191.

[8] Carrel, A., *J. Exptl. Med.,* **34** (1921): 333.

LITERATURE CITED

Akamatsu, N., *Virchow's Arch. Path. Anat.,* **240** (1923): 308.

Carrel, A., *J. Exptl. Med.,* **15** (1912): 393; **34** (1921): 333.

Cook, E. S., and Kreke, C. W., THIS ISSUE, p. 47.

Drew, A. H., *Brit. J. Exptl. Path.,* **4** (1923): 46.

Fardon, J. C., and Sullivan, W. A., THIS ISSUE, p. 39.

Lewis, W. H., and Lewis, M. R., General Cytology, University of Chicago Press, 1924, p. 385.

Loofbourow, J. R., and Dwyer, Sr. Cecelia Marie, S.C. *Science,* **88** (1938): 191.

Original Manuscript submitted
October 11, 1938

LOCAL ANESTHETICS.
THE RELATION OF CHEMICAL STRUCTURE TO PHYSIOLOGICAL ACTION IN THE AMINOESTER SERIES

BY ELTON S. COOK

LOCAL anesthesia of a sort has been used from very early times. As far back as 2500 B.C. a local insensitivity to pain was obtained by pressure on a nerve trunk; the same method was still in use during the eighteenth and early nineteenth centuries.[1] Dioscorides mentions the topical application of plant extracts. Rose oil (containing phenylethyl alcohol) was used for painful eye affections. In later times cold was employed. In 1849 James Arnott used ice and salt and Richardson introduced the evaporation of ether for this purpose in 1867.[2] The cold produced by the rapid evaporation of ethyl chloride is still used in certain minor procedures.

Cocaine. Local anesthesia in the modern sense began with the discovery of cocaine. The aborigines of Peru chewed coca leaves and thereby experienced a feeling of exhilaration which enabled them to do an abnormal amount of work. They were also familiar with the use of the juice of the coca leaf to obtain insensitivity to pain. It is probable that the Incas used the active principle of coca leaves in trephining.[3] News of these remarkable properties was brought to Europe by the Spaniards as early as the sixteenth century but no practical results issued from this knowledge until towards the end of the nineteenth century. Wöhler obtained a quantity of coca leaves and turned them over to Niemann, who, in 1859-60, isolated the active principle in pure form and named it cocaine.[4] The latter observed that cocaine had a numbing effect on the tongue. This property was confirmed by Demarlé and Schraff in 1862. In 1868 Moreno y Maiz published a monograph in which were stressed the anesthetic properties of cocaine on the peripheral nerves,[5] but this apparently attracted little attention. The real clinical use of cocaine dates from September 15, 1884 when Karl Koller, at the suggestion of Freud, investigated it and discovered its remarkable effectiveness in ophthalmology. By December 6 of the same year, Halsted, in the United States, had used cocaine for nerve block, and from that time on its use grew.

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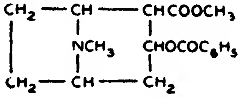
[1] Singer, A Short History of Medicine, New York (1928):26. For the early history of local anesthesia see Braun, Die örtliche Betäubung, Leipzig, 6th ed. (English translation of the third edition by Shields, Phila., 1914.)

[2] Munch, Bioassays, Baltimore (1931): 54.

[3] Mozans, Along the Andes and Down the Amazon, New York (1911): 206, 207.

Work by several investigators culminated in the establishment of the constitution of cocaine in 1901-3 by Willstätter,[6] who showed it to have the following structure:



This structure was confirmed by brilliant synthetic work. Willstätter's first synthesis involved twenty-seven major steps; his second, seven; and his final synthesis, which he patented, three.[7]

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[4] Niemann, *Ann.* **140** (1860): 213.

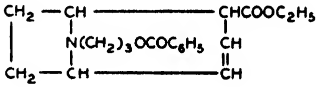
[5] Moreno y Maiz, Thèse de Paris (1868). *Cf.* von Oettingen, *Ann. Med. Hist.* **5** (1933): 275.

[6] Willstätter, Wolfes and Mäder, *Ann.*, **434** (1923): 11, for account of this work.

[7] *Cf.* Fränkel, Die Arzneimittel-Synthese, Berlin (1927): 354-357.

It was shown[8] that the unbenzoylated and unmethylated nucleus, ecgonine, was without anesthetic activity; and if either the benzoyl group or the methyl group were removed, the resulting compound was also inactive.

Cocaine Type—Cyclic Compounds. Many modifications of cocaine were made without destroying the essential cyclic structure. Esterifying groups other than benzoyl were almost uniformly unsuccessful.[9] Oxidation of ecgonine with potassium permanganate removed the nitrogen methyl[10] and the corresponding nitrogen demethylated cocaine (norcocaine) was found to be very active but more toxic than cocaine.* One of the interesting synthetics derived from cocaine was eccaine with the following structure:[11]



Here the important benzoyl group is joined to the nitrogen by a three-carbon straight chain rather than by a three-carbon portion of the cycle. This will prove to be an important development later.

Atropine is very closely related to cocaine in structure and has, in itself, a weak local anesthetic action.[12] It is an ester of tropine with tropic acid:

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[8] Merck, *Ber.*, 18 (1885): 2954; 21 (1888): 48; Novy, *Am. Chem. J.*, 10 (1888): 147.

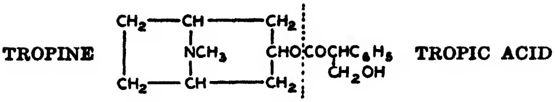
[9] *Cf.* Fränkel, *op. cit.*, pp. 348-350.

[10] Einhorn, *Ber.*, 21 (1888): 3029, 3411.

*A valuable table of ecgonine derivatives is to be found in von Oettingen, The Therapeutic Agents of the Pyrrole and Pyridine Groups, pp. 206-7.

[11] von Braun and Müller, *Ber.*, 51 (1918): 235.

[12] Filehne, *Berlin. klin. Wochschr.* (1887): 107.



If benzoic be substituted for tropic acid, the resulting compound, tropacocaine, possesses stronger local anesthetic properties than cocaine.[13]* Interestingly enough, this compound occurs naturally in Java coca leaves.[16]

It is desirable, if possible, to simplify the cocaine molecule. If part of the molecular superstructure can be discarded without loss—and perhaps with gain—both the academic problem of relating physiological activity to chemical structure and the practical problem of producing a better local anesthetic will be furthered. It must be remembered that cocaine is far from ideal as a local anesthetic. Its habit forming properties and toxicity count greatly against it, and compared with many modern anesthetics, its activity is relatively low. In addition, its solutions cannot be sterilized by boiling without considerable decomposition.

Among the most successful of the earlier attempts at simplification, while retaining the cyclic structure, were α-eucaine[17] and β-eucaine.[18] In these compounds the two carbon atoms which may be regarded as forming a pyrrolidine ring in cocaine have been discarded without loss.

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*Tropacocaine, properly speaking, is the benzoyl ester of pseudotropine, a stereoisomer of tropine.[14] The benzoate of tropine itself is a weaker anesthetic. Tropine is converted to pseudotropine by heating with sodium amylate.[15] Similarly, cocaine is transformed to the stereoisomer pseudococaine by heating with alkali. The d-form of pseudococaine is on the market as psicaine.

[13] Chadbourne, *Brit. Med. J.* (1892): 402.

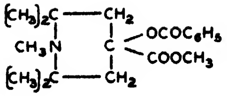
[14] Liebermann, *Ber.*, 24 (1891): 2336, 2587; 25 (1892): 927.

[15] German Patent 88,270.

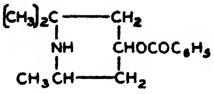
[16] Giesel, *Pharm. Ztg.* (1891): 149.

[17] Merling, *Ber. deut. pharm. Ges.*, 6 (1897): 173.

[18] German Patent 90,069.



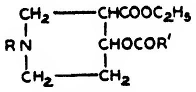
α—EUCAINE



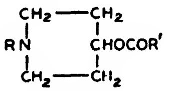
β—EUCAINE

The remaining essentials are a piperidine ring and an ester group. α-Eucaine, while less toxic than cocaine, is more toxic than β-eucaine and is somewhat irritating. β-Eucaine has been more successful in practice.[19] It is of interest that α-cocaine, the cocaine analog of α-eucaine, is devoid of anesthetic properties.[20]

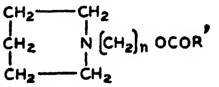
This type of piperidine compound has recently been extensively investigated, chiefly by McElvain,[21] who has not only increased our knowledge of local anesthetics but has also improved our methods of synthesis in the piperidine series. His compounds consist mostly of the following types:



TYPE I



TYPE II



TYPE III

In these, R is generally a simple alkyl and R′ is $—C_6H_5$, $—C_6H_4NH_2(p)$, or $—CH=CHC_6H_5$. In the first two types an increase in activity and decrease in toxicity was found as the size of R became greater. Of the ester groups, *p*-amino benzoyl was both more active and more toxic than benzoic. Type III has given rise to one practical anesthetic, metycaine, and will be considered later.

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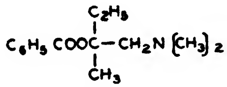
[19] Vinci, *Arch. path. Anat.* (*Virchow's*), 149 (1897): 201.

[20] Willstätter, *Ber.*, 29 (1896): 1575, 2216.

[21] McElvain, *J. Am. Chem. Soc.*, 46 (1924): 1721; 48 (1926): 2179, 2239; 49 (1927): 2835; Thayer and McElvain, *ibid.*, 49 (1927): 2862; 50 (1928): 3348; Bolyard and McElvain, *ibid.*, 51 (1929): 922; Bailey and McElvain, *ibid.*, 52 (1930): 1633, 2007. *Cf.* also Pyman, *J. Chem. Soc.*, 93 (1908): 1793; Brill, *J. Am. Chem. Soc.*, 47 (1925): 1134; Barnes and Adams, *ibid.*, 49 (1927): 1307; Brit. Pat. 241, 767 (*Chem. Abst.*, 20 [1926]: 3539); Sandborn and Marvel, *J. Am. Chem. Soc.* 50 (1928): 563; Marvel and Shelton, *ibid.*, 51 (1929): 915; Blicke and Blake, *ibid.*, 53 (1931): 1015.

Straight Chain Compounds. In the work with cocaine and related compounds it was shown that part of the molecule could be destroyed without loss of anesthetic properties. The essential parts seemed to be an ester group and a piperidine ring. It appears reasonable that the piperidine ring might be replaced by acyclic amines. We have already had hints of this possibility in eccaine and in the work of McElvain *et al.* We may, then, suggest that the important parts of a local anesthetic are an ester group separated from an amino group by a carbon chain. Indeed, von Braun and Müller in their paper on eccaine[11] present considerable evidence that the physiological activity of cocaine depends upon an acylated hydroxyl in the γ-position to nitrogen.

Fourneau coincided with this opinion and believed that cocaine action is derived from a secondary or tertiary amino group and an alcohol group, preferably tertiary. This led to the synthesis of a large number of compounds[22] of which the best was stovaine.[23] Stovaine is less toxic than cocaine[24] and has found special use as a spinal anesthetic.



$$C_6H_5COO\overset{C_2H_5}{\underset{CH_3}{C}}-CH_2N(CH_3)_2$$

STOVAINE

Substitution of another dimethyl amino group in stovaine produces alypine[25] which is both more basic and more toxic than stovaine.

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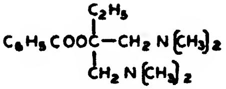
[22] For a summary of much of Fourneau's work, see Fränkel, *op. cit.*, pp. 379-384.

[23] Fourneau, *Compt. rend.*, 138 (1904): 7661.

[24] Smith and Hatcher, *J. Pharmacol.*, 9 (1917): 231.

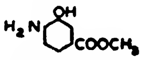
[25] Bayer, German Patent 173,631; Impens, *Arch. ges. Physiol.* (*Pflügers*), 110 (1905): 21.



$C_6H_5COO\overset{C_2H_5}{\underset{CH_2N(CH_3)_2}{C}}-CH_2N(CH_3)_2$

ALYPINE

Procaine. The greatest advance in synthetic local anesthetics came with the discovery of procaine (novocaine) by Einhorn[26] in 1905. This discovery resulted not only from the work in cocaine chemistry but also from a knowledge of the local anesthetic properties of a number of simple esters. Ritsert,[27] in 1890, prepared ethyl para-aminobenzoate, $p\text{-}NH_2C_6H_4COOC_2H_5$ (anesthesin) and noticed that it had topical anesthetic activity.[28] This discovery went unnoticed for many years and ethyl para-aminobenzoate was not introduced to the trade until 1902. When this series was further investigated, it was noticed that local anesthetic activity increased as the size of the ester group increased. The butyl ester is in use today in ointments as butesin. Meanwhile, in 1896-7, Einhorn introduced orthoform into therapy.[29] In orthoform it was found that the introduction of a hydroxyl group into the benzene nucleus enhanced the local anesthetic activity.



ORTHOFORM

An isomer of orthoform, called New Orthoform, was produced, and still another compound of this type that has had some use is nirvanine. All of these simple esters have one great disadvantage, that of low water solubility. This unfortunate property has largely prevented the use of these compounds except as dusting powders or in ointments.

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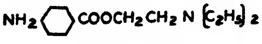
[26] Einhorn and Uhlfelder, *Ann.*, 371 (1909): 131; U. S. Patent 812,554.

[27] Ritsert, *Pharm. Ztg.*, 70 (1925): 1006.

[28] Bodendorf, *Deut. Apoth. Ztg.*, 49 (1934): 1642.

[29] Burnett, Jenkins, Peet, Dreger and Adams, *J. Am. Chem. Soc.*, 59 (1937): 2248.

In procaine Einhorn produced a simple, water-soluble compound. Procaine is β-diethylaminoethyl-para-aminobenzoate. It is most simply prepared by



PROCAINE

reacting diethylamine with ethylene chlorohydrin, esterifying the resulting β-diethylaminoethanol with para-nitrobenzoylchloride and reducing. Procaine is today probably the most widely used compound for injection and spinal anesthesia, this wide use being favored by its relatively low toxicity. It is, however, notoriously lacking in activity when applied topically (direct to mucous membrane surfaces). In spite of its disadvantages, cocaine remained for a long time the only available topical anesthetic. Many attempts have been made to find a compound valuable as an anesthetic, both when used topically or by injection, of greater activity than procaine, and of low toxicity.

Straight Chain Aminoester Anesthetics. A host of local anesthetics have been prepared by slight modification of the fundamental procaine structure. For the most part these modifications have been of three types: (1) the substitution of different groups on the amino alcohol nitrogen; (2) variation of length of the alcohol chain and branching of the chain; and (3) the esterification of amino alcohols with acids other than benzoic or para-aminobenzoic. We shall consider first a few of the compounds which differ chiefly by modification of the amino alcohol. An excellent study of various amino alcohol esters of *p*-aminobenzoic acid has recently appeared.[29]

Einhorn[30] himself investigated the effect of various alkyls substituted on the nitrogen. Adams and co-workers[29,31] studied a series of γ-dialkyl-aminopropanol para-aminobenzoates differing from the procaine series only in that the alcohol chain was one carbon longer,

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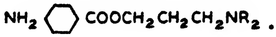
[30] Einhorn, Fiedler, Ladisch and Uhlfelder, *Ann.*, **371** (1909): 142.

[31] Kamm, Adams, and Volwiler, U. S. Patents, 1,358,750 and 1,358,751; Volwiler, *Science*, **53** (1921): 145.



Both the ethanol and propanol series were studied pharmacologically by Schmitz and Loevenhart[32] who arrived at the following conclusions, which have generally been substantiated by other workers with similar series. In both series there is an increase in local anesthetic activity (topical) and toxicity as the size of the nitrogen alkyl increases. It is of considerable interest that an isopropyl group is superior to *n*-propyl. The propanol derivatives are in all cases more active and more toxic than the corresponding ethanol homologs. Thus, both an increase in the length of the carbon chain and in the size of the alkyl increases the physiological activity. However, the toxicity and anesthetic efficiency may not increase at the same rate so that, in individual cases, a favorable ratio of minimum lethal to minimum effective dose may occur. These generalizations have been confirmed recently by Adams in more extensive series.[29] Cyclic amino alcohols, such as dialkylaminocyclohexanols, behave similarly.[33] Here, too, an increase in the effective carbon chain, i.e., the number of carbon atoms between the amino and carboxyl groups, increases the local anesthetic activity to a maximum at three or four carbon atoms, depending upon the method of testing. This effect of increase in length of the alcohol chain was corroborated by Adams *et al.*[34] in a series of simple alkyl para-aminobenzoates of the anesthesin type:

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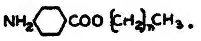
[32] Schmitz and Loevenhart, *J. Pharmacol.*, 24 (1924): 159.

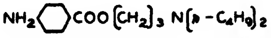
[33] Mannich and Braun, *Ber.*, 53 (1920): 1874; Osterberg and Kendall, *J. Am. Chem. Soc.*, 43 (1921): 1370; Heckel with Adams, *ibid.*, 49 (1927): 1303.

[34] Adams, Rideal, Burnett, Jenkins and Dreger, *J. Am. Chem. Soc.*, 48 (1926): 1758.



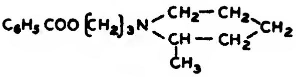
An increase in activity parallels an increase in length of carbon chain to butyl; increasing the number of carbons beyond four has only a very slight effect, probably due, at least in part, to the greater insolubility as the molecular weight rises. Branching of the alcohol chain generally decreases anesthetic power in this simple series. However, branching of the alcohol chain in a series of alkyl esters of *p*-aminophenyl carbamic acid increased the local anesthetic activity and decreased the toxicity, and secondary alcohols gave less active esters.[56]

A practical result of this research was butyn, γ-di-*n*-butylaminopropanol-*p*-aminobenzoate. Butyn has had considerable use in ophthalmology.



BUTYN

Mention should be made at this point of metycaine, the outcome of McElvain's work previously discussed. It is the benzoate of γ-(2-methylpiperidino) propanol.



METYCAINE

Formerly known as neothesin, it has recently been put on the market and has been used for topical, injection, and spinal anesthesia.

Pontocaine (or pantocaine)[35] is also a commercial anesthetic of interest because of the butyl substituted *p*-aminobenzoic acid used. This substitution seems to increase the activity, whereas, it will be recalled,

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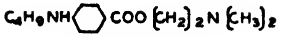
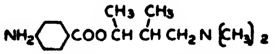
[35] Fussgänger and Schaumann, *Arch. exptl. Path. Pharmakol.*, **160** (1931): 53; Ernst, *Münch. med. Wochschr.*, **128** (1931): 9.



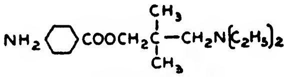
PONTOCAINE

the opposite is usually the case in the insoluble ester series (anesthesin type). In spite of a rather high toxicity, pontocaine has found use in spinal anesthesia. Some theoretical implications of its structure will be discussed later.

The effect of branching the alcohol chain in simple alkyl esters has been mentioned above and seems to vary with the acid used. In the amino-alkyl ester series (esters of amino alcohols) branching the chain generally increases the local anesthetic activity. Much work has been done with branched chain alcohols and two anesthetics of this type are on the market. These are tutocaine[36] and larocaine[37] and they have the following structures:

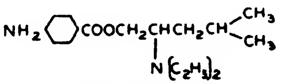


TUTOCAINE



LAROCAINE

Tutocaine has been particularly useful in lumbar anesthesia while larocaine is being advocated as a topical anesthetic. Both anesthetics are in wider use in Europe than in the United States. Their structural significance will be discussed later. Another anesthetic of this type, panthesine, was synthesized by Karrer.[38] It is the *p*-aminobenzoate of N-diethylaminoleucinol (iso-capryl alcohol).



PANTHESINE

In passing, we have seen that the amine of the amino alcohol is usually a simple dialkylamino or piperidino group.

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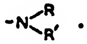
[36] U. S. Patent 1,474,576. *Cf.* Schulemann, *Klin. Wochschr.*, 3 (1924): 676.

[37] Fromherz, *Arch. exptl. Path. Pharmakol*, 158 (1930): 368.

[38] Karrer, *Helv. chim. Acta.*, 4 (1921): 92. *Cf.* Berret, Thèse, Geneva (1931).

A study of the effect of the purity of the piperidine on certain local anesthetics was made by Cook and Rider[39] and it was found that, at least in some cases, anesthetics derived from piperidine and 2-methyl piperidine are physiologically antagonistic. A series of monoalkylamino alcohol esters has recently been prepared[40] and some of these compounds have promise.

Of obvious interest is the use of dissimilar alkyls on the nitrogen,


$$-N\begin{matrix}\nearrow R\\ \searrow R'\end{matrix}\ .$$

In fact, the piperidino group may be considered as a sort of cyclized propylethylamine. A number of compounds of this type have been made, for example, by von Braun,[41] Thorp,[42] Volwiler and Adams,[43] McElvain,[44] and Brill.[45] Usually these compounds have not been outstanding, and a practical hindrance to their study is found in the difficulty and cost of preparation of unsymmetrical amines.

Aromatic amines seem to be undesirably toxic, but when the aromatic group is separated from the nitrogen by a carbon chain this toxicity is diminished but not entirely obviated. Examples of this type may be found in several of the mixed amino compounds mentioned in the preceding paragraph and especially in the paper of Cope and McElvain.[44] In this connection the attachment of the nitrogen to a reduced benzenoid ring system is of interest. The use of amino cyclohexanols[33] has been mentioned. *Ac*-tetrahydronaphthalene amino alcohols have been investigated by Cook and Hill,[46] who have prepared esters of 2-dialkylamino-3-hydroxy- and 1-dialkylamino-2-hydroxy-1,2,3,4-tetrahydronaphthalenes which possessed considerable local anesthetic activity. Recently Coles and Lott[47] synthesized active derivatives of *ac*-*β*-tetrahydronaphthylamine, and Mannich[48] has prepared esters of 1-hydroxy-2-(dialkylaminomethyl)-1,2,3,4-tetrahydronaphthalenes.

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[39] Cook and Rider, *J. Am. Chem. Soc.*, 59 (1937): 1739; Rider and Cook, *ibid.*, 59 (1937): 1741; Cook, *ibid.*, 59 (1937): 2661; Rider and Cook, *J. Pharmacol.*, 64 (1938): 1.

[40] Goldberg and Whitmore, *J. Am. Chem. Soc.*, 59 (1937): 2280; Abramson and Goldberg, *J. Pharmacol*, 62 (1938): 69.

[41] von Braun and Kirschbaum, *Ber.*, 52 (1919): 2011; von Braun and Braunsdorf, *ibid.*, 54 (1921): 2081.

[42] Thorp, U. S. Patent 1,193,634.

[43] Volwiler and Adams, U. S. Patent 1,476,934; Burnett, Jenkins, Peet, Dreger and Adams, *J. Am. Chem. Soc.*, 59 (1937): 2248.

[44] Cope and McElvain, *J. Am. Chem. Soc.*, 53 (1931): 1587.

[45] Brill, *ibid.*, 54 (1932): 2484.

The use of heterocyclic amines other than piperidine has been studied by several investigators. Among the heterocycles may be mentioned piperazine,[49] morpholine,[50] and thiomorpholine.[51] Much further study could be done in this field.

We have considered the amino alcohol portion of the local anesthetic molecule but have thus far only given cursory attention to the acidic moiety. As we have seen, the majority of local anesthetics in active use are esters of benzoic or para-aminobenzoic acid. Which acid is preferable seems to depend upon the individual compound in hand. Thus, in many cases the para-aminobenzoates are both more active and less toxic than the corresponding benzoates, but this is not always true. For example, Cope and McElvain,[44] in one lengthy series of compounds, found the benzoates to be irritating, of low toxicity, and of highest activity when attached to a three-carbon alcohol chain. The corresponding aminobenzoates were non-irritating, had a high toxicity, and gave the most active anesthetics when attached to a two-carbon alcohol chain.

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[46] Cook and Hill, to be published. *Cf.* Cook, Dissertation, Yale University (1933).

[47] Coles and Lott, *J. Am. Chem. Soc.*, **58** (1936): 1989.

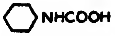
[48] Mannich, Borkowsky and Lin, *Arch. Pharm.*, **275** (1937): 54.

[49] Pyman, *J. Chem. Soc.*, **93** (1908): 1802; Gardner and Schneider, *J. Am. Chem. Soc.*, **55** (1933): 3823; Adelson, MacDonald and Pollard, *ibid.*, **57** (1935): 1988.

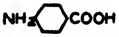
[50] (a) Leffler and Brill, *J. Am. Chem. Soc.*, **55** (1933): 365; (b) Gardner, Clark and Semb, *ibid.*, **55** (1933): 2999; (c) Gardner and Hammel, *ibid.*, **58** (1936): 1360.

[51] Burrows and Reid, *ibid.*, **56** (1934): 1720; Macht and Burrows, *Am. J. Physiol.*, **113** (1935): 92.

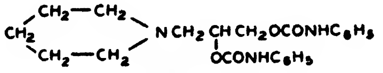
A great many other acids have been investigated. Of these, one of the most interesting is phenylcarbamic. Phenylcarbamic acid is isomeric with aminobenzoic but has the NH_2 group covered and directly attached to the carboxyl. Early work[52] with phenyl carbamates (or phenylurethans) led to the belief that they had rather unsatisfactory properties. The field was reopened by Rider, who studied the phenylurethans of dialkylaminopropanediols.[53] One of the compounds investigated is now on the market as Diothane (piperidinopropanedioldiphenylurethan).[54] This compound has excellent topical anesthetic properties which have made it especially useful in urology. It is unique in that it gives a prolonged period of analgesia, sometimes lasting a day or more, after surgical anesthesia has worn off. Phenylurethans have been further investigated by Cook and Rider[55] who have concluded that the phenylurethan



PHENYLCARBAMIC ACID



p–AMINOBENZOIC ACID



DIOTHANE

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[52] Fromherz, *Arch. exptl. Path. Pharmakol.*, 76 (1914): 257; Bonar and Sollman, *J. Pharmacol.*, 18 (1921): 467.

[53] Rider, *J. Am. Chem. Soc.*, 52 (1930): 2115, 2583; *J. Pharmacol.*, 39 (1930): 457; *ibid.*, 47 (1933): 255.

[54] Rider and Cook, *J. Pharmacol.*, 64 (1938): 1.

[55] Cook and Rider, *J. Am. Chem. Soc.*, 58 (1936): 1079; Rider and Cook, *ibid.*, 59 (1937): 1741.

group, as compared with the isomeric *p*-aminobenzoate, confers conspicuous topical anesthetic activity upon the molecule. These authors found that the phenylurethans of secondary propyl alcohols showed higher activity than the esters of the corresponding primary alcohols.

Para-aminophenylurethans are active but irritating.[56] Some mixed phenylnaphthyl urethans have also been studied.[57] Some nitrogen carboxylated carbazoles[58] can properly be considered as urethans, as can similarly carboxylated pyrroles.[59]

Another interesting variation has been the substitution of *p*-aminothiobenzoic acid for *p*-aminobenzoic in procaine.[60] The resulting thioprocaine (thiocaine) is both more active and more toxic than procaine. Further work in this series has been done by others.[61]

Of the 2 per cent of alkaloids in the coca plant, about 1 per cent contains the cocaine nucleus esterified with acids other than benzoic. Of these other acids the simplest is cinnamic. Cinnamic acid has been esterified with many amino alcohols[62] to produce local anesthetics. One cinnamic ester, apothesine,[63] is on the market.

$$C_6H_5CH = CHCOOCH_2CH_2CH_2N(C_2H_5)_2$$

APOTHESINE

The substitution of other aromatic rings for the benzene ring in benzoic acid has interesting possibilities. Hill and coworkers[64] have investigated the naphthoic esters, simple and substituted, of amino alcohols. Of these, the best was found to be 2-ethoxy-3-(γ-diethylaminopropyl) naphthoate,

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[56] Horne, Cox and Shriner, *ibid.*, **55** (1933): 3435.

[57] Boese and Major, *ibid.*, **57** (1935): 175.

[58] Knoefel, *J. Pharmacol.*. **47** (1933): 69.

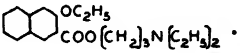
[59] Andrews and McElvain, *J. Am. Chem. Soc.*, **51** (1929): 887.

[60] Hansen and Fosdick, *ibid.*, **55** (1933): 2872; Fosdick and Hansen, *J. Am. Dental Assoc.*, **21** (1934): 1777; Fosdick and Hansen, *J. Pharmacol.*, **50** (1934): 323.

[61] Karjala and McElvain, *J. Am. Chem. Soc.*, **55** (1933); 2966; Lischer and Jordan, *ibid.*, **59** (1937): 1623.

[62] *Cf.* Brill and Cook, *J. Am. Chem. Soc.*, **55** (1933): 2062.

[63] Wildman and Thorp, U. S. Patent 1,193,649.



Acids derived from the other aromatic nuclei, pyrrole,[65,66] thiophene,[66] and furan,[66] have also been studied and they all seem to be less satisfactory than benzene, arranging themselves in the following order of efficiency:[66]

Benzene > pyrrole > thiophene > furan.

However, an exception is found in the simple alkyl furoates which are considerably more active than the corresponding benzoates.[67] Further work on furan compounds has recently been carried out by Walter.[67a]

One of the important recent developments has been the use of the quinoline nucleus in the form of cinchoninic acids. The value of this nucleus might be suspected since quinine and its closely related derivatives have local anesthetic properties, as will be discussed later. The outstanding quinoline local anesthetic is nupercaine, α-butoxycinchoninic acid diethylaminoethylene amide, discovered by Miescher.[68] Although nuper-

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[64] Robinson and Hill, Organic Chem. Symposium, Columbus, Ohio (Dec., 1927); Smith, Dissertation, Yale University (1929); Fisk and Underhill, *J. Pharmacol.*, 49 (1933): 320. *Cf.* also Bjerregaard and Houstin, *Proc. Okla. Acad. Sci.*, 14 (1934): 77.

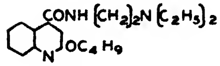
[65] Blicke and Blake, *J. Am. Chem. Soc.*, 52 (1930): 235; *ibid.*, 53 (1931): 1015.

[66] Gilman and Pickens, *ibid.*, 47 (1925): 245.

[67] Phatak and Emerson, *J. Pharmacol.*, 58 (1936): 174.

[67a] Walter, *J. Am. Chem. Soc.*, 60 (1938): 2467.

[68] (a) Miescher, U. S. Patent 1,688,469 (1928); (b) Miescher, *Helv. chim. Acta*, 15 (1932): 163; (c) *Cf.* von Oettingen, The Therapeutic Agents of the Quinoline Group, New York (1933), pp. 100-105.



NUPERCAINE

caine is an amide rather than an ester, it may be discussed here since it has been shown[69] that the ester group can replace the amide group without essential change of properties. The significance of the amide group will be treated later. Although very powerful, its high toxicity renders nupercaine potentially dangerous. None the less, nupercaine enjoys wide use, especially in topical and spinal anesthesia.

The alkoxyl group plays an important part in the potency of nupercaine, the duration of anesthesia rising with increasing length of the chain to a maximum at four carbon atoms and then dropping.[68b] Hill[64] has shown that the introduction of alkoxyl groups into the naphthalene nucleus also increases local anesthetic activity. Hence, it would appear that the introduction of these groups into other series of anesthetics would be worthy of study.

The esterification of alkoxycinchoninic acids with morpholine amino alcohols has recently been reported.[50c]

The use of aliphatic rather than aromatic acids has not been very thoroughly investigated, probably because of the generally disappointing results obtained with those which have been studied.[70] Aliphatic acids increase in potency with increase in molecular weight but this beneficial effect is offset by a decrease in solubility. Aromatic-substituted aliphatic acids, such as phenylacetic and phenylpropionic, have also been generally unsatisfactory[71] as has *p*-aminomandelic.[71a] In a series of unsaturated aliphatic acids, Bachman[72] pointed out that local anesthetic power is inversely proportional to the degree of unsaturation of the acid. β′-Diethylaminoethyl-β-aminocrotonate is inactive.[73] However, when an unsaturated group is placed between the phenyl and carboxyl, as in cinnamic acid (apothesine), activity is secured.[74] This seems to be the reverse of the behavior of aliphatic acids but is in keeping with a more or less general rule that unsaturation increases the physiological activity (including toxicity) of compounds. However, when a triple bond is substituted for the double bond of cinnamic acid (phenylpropiolic acid), local anesthetic activity is lost.[74b]

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[69] Wojahn, *Arch. Pharm.*, **269** (1931): 422.

[70] *Cf.* Brill and Bulow, *J. Am. Chem. Soc.*, **55** (1933): 2059.

[71] Pyman, *J. Chem. Soc.*, **111** (1917): 167, 1119; Kurvahata, Ochiai and Nukita, *Folia Pharmacol. Japon.* **7** (1928): 408.

[71a] Fosdick and Wessinger, *J. Am. Chem. Soc.*, **60** (1938): 1465.

It would thus appear that the most satisfactory type of acid is aromatic in which the carboxyl is directly attached to the aromatic group, or attached to it via an unsaturated group or nitrogen atom (phenylurethan).

SUMMARY OF THE RELATION BETWEEN CHEMICAL CONSTITUTION AND LOCAL ANESTHETIC ACTIVITY IN THE ESTER SERIES.

We have seen that simple aromatic esters (anesthesin type) have local anesthetic activity but that this activity is considerably increased when an amino alcohol replaces the simple alcohol. Hence, we are led to suspect that the anesthetic activity resides in the ester portion of the molecule and that the amino group plays a supplementary, but important, part. This division of a physiologically active molecule was first applied in other fields by Ehrlich in his pharmacophore theory, a counterpart of the chromophore theory of dye chemistry. According to this theory, the ester portion of the molecule, which is active in producing anesthesia, is the pharmacophore, or specifically, the anesthesiophore group. The remainder of the molecule is a basic anchoring group by means of which the molecule is attached to the tissue. Ehrlich applied this theory to cocaine in 1890,[75] and it was extended to local anesthetics generally by Fourneau.[76]

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[72] Bachman, *J. Am. Chem. Soc.*, 57 (1935): 2167.

[73] Shriner and Keyser, *J. Am. Chem. Soc.*, 60 (1938): 286.

[74] (a) Kamm, *J. Am. Chem. Soc.*, 42 (1920): 1030; (b) Gilman and Pickens, *ibid.*, 47 (1925): 245; *ibid.*, 50 (1928): 437.

Considering first the amino alcohol moiety of the molecule, a straight, branched, or cyclic carbon chain may be used. The activity and toxicity increase with the length of the carbon chain (number of carbon atoms between nitrogen and hydroxyl) to a probable maximum at four or five carbons. For practical purposes (high activity, low toxicity, sufficient solubility, and low cost) a carbon chain of two or three atoms seems best. The effect of branching the alcohol chain is variable and should be investigated further. The alcohol may be primary, secondary, or tertiary. Fourneau preferred tertiary,[76] but here, again, the activity varies with different series of anesthetics. The amine may be cyclic or not and has usually been tertiary; secondary amino groups are active but are more difficult to prepare. In a dialkyl amine the alkyls may be similar or not. Aromatic amines generally cause an undue increase in toxicity, but aromatic substituents on aliphatic chains may be employed and appear to increase both activity and toxicity.

The acid portion of the molecule is preferably aromatic. The aromatic group should be attached directly to the carboxyl or via an unsaturated group (cinnamic acid)[74a] or nitrogen. The last type (phenylurethan) seems specifically to increase the surface anesthetic activity. Such work as has been done suggests that the substitution of alkoxyl groups, especially butoxyl, on the aromatic ring enhances the anesthetic effect.

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[75] Ehrlich, *Deut. med. Wochschr.*, **16** (1890): 717.

[76] Fourneau, *J. pharm. chim.*, **2** (1910): 56.

Viewing the aminoester molecule as a whole, it seems to present, at least partially, a problem in balanced basicity. Fenwick and Gilman[77] have found local anesthetic activity to increase with increasing basicity of the molecule as measured by the basic dissociation constant, but Adams *et al.*[34] have shown that this is not an invariable rule. It can be pointed out that an increase in the size of the nitrogen alkyls and the branching of the alcohol chain decrease the basicity while an increase in the length of the alcohol chain increases the basicity of the molecule.[78] The use of aminobenzoic in place of benzoic acid increases the basicity but substitution of the amino group of aminobenzoic acid, as in pontocaine, decreases it. Examination of the formulas of many local anesthetics (cf. pontocaine, tutocaine, larocaine, and panthesine in particular) reveals much manipulation of the basicity, increasing it here, decreasing it there, so that a proper total effect is achieved. These results, however, are largely empirical and it must be admitted that we know very little about the real issues involved.

Aminoester Salts. One aspect of aminoester local anesthetics has been neglected thus far in this review and must be considered now. In actual practice the aminoesters are not used as such because, generally, they are insufficiently soluble. Hence, they are reacted with acids to form soluble salts, $RCOO(CH_2)_nNR_2.HX$, and it is these salts which are actually employed. It has long been considered that the acid (usually a strong mineral acid such as hydrochloric or sulfuric) merely exerted a solubilizing influence. The local anesthetic action was due to the aminoester portion (free base) of the salt alone, and this free base was released by hydrolysis of the salt. The free base was supposed to be preferentially soluble in the lipoid portion of the cells according to the classic Meyer-Overton concept of anesthesia.[79] This theory of action was originally proposed by Gros[80] and was supported by the fact that alkalinizing a local anesthetic solution increased its activity. It led to the use of weak acids, such as boric,[81] since the borate would be more completely hydrolyzed than, say, the hydrochloride, and would furnish a higher concentration of free base.

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[77] Fenwick and Gilman, *J. Biol. Chem.* 84 (1929): 605.

[78] Vliet and Adams, *J. Am. Chem. Soc.*, 48 (1926): 2158.

This theory was widely accepted and is, indeed, the prevalent theory today. However, certain facts militate against it. Régnier[82] actually determined the anesthetic activity of cocaine free base and of its alkalinized hydrochloride and showed that the latter had an activity four times that of the free base. This suggested some specific activity on the part of the alkali. Continuing along this line, Régnier and David[83] experimented with cocaine dissolved in buffer solutions and found that acetate and phosphate buffers of the same pH produced remarkable differences in length of anesthesia. This pointed to a specific effect of the salt since solutions of the same pH would be expected to contain equal quantities of free base. These authors then prepared a series of thirteen salts of cocaine free base and found that these differed greatly in activity, ranging from the citrate, which is 0.2 as powerful as cocaine hydrochloride, to the phenyl acetate, which is twelve times as active.[84] An important result of these experiments was the observation that the anions, when arranged in order of anesthetic activity, fell into the order of the Hofmeister series and not into the order of degree of hydrolysis as would be demanded by the Gros theory. Régnier has followed this work with a series of procaine salts and finds similar results.[85] These findings point to the importance of the protein portion of the cell which was largely neglected by the Meyer-Overton theory. The importance of the combination of local anesthetics with protein as influencing the fixation of the anesthetic was indicated long ago by Moore and Roaf[86] and more recently by Rider.[87] Régnier concludes that the acid may act by affecting the rate of absorption or fixation in the cell; and if one does not separate penetration from local anesthetic activity it is possible to conclude, as Rider has suggested,[88] that the anesthetic acts as the undissociated salt and not as the free base or ion. The relative activity of a series of salts then depends upon which is most acceptable to the cell. Studies of Dietzel and Steeger[89] on the hydrolysis of several cocaine salts during sterilization may give some support to the results of Régnier. Unfortunately Moore[89a] has been unable to confirm Régnier's results, and the present author, in unpublished experiments, could not demonstrate salt effects approaching the magnitude of those found by Régnier. The status of the problem is therefore uncertain.

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[79] Meyer, *Arch. exptl. Path. Pharmacol*, **46** (1901): 338; Overton, Studien über die Narkose, Jena (1901).

[80] Gros, *Arch. exptl. Path. Pharmakol.*, **62** (1910): 380; *ibid.*, **63** (1910): 80; *ibid.*, **67** (1912): 126; *Cf.* Hirschfelder and Bieter, *Physiol. Rev.*, **12** (1932): 190, for discussion.

[81] *Cf.* Copeland and Notton, *Brit Med. J.*, **2** (1925): 547.

[82] Régnier, *Bull. sci. pharmacol*, **31** (1924): 513; *ibid.*, **32** (1925): 271.

[83] Régnier and David, *ibid.*, **41** (1934): 321.

[84] Régnier and David, *Compt. rend.*, **200** (1935): 1428; *Anesthésie et Analgésie*, **1** (1935): 285; *J. pharm. chim.*, **22** (1935): 16.

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[85] Régnier, Delange and David, *Compt. rend.*, 202 (1936): 591; Régnier, French Patent 789,156; Régnier and Quevauviller, *Compt. rend. soc. biol.*, 122 (1936): 251; Régnier, Lambin and Szollosy, *ibid.*, 122 (1936): 759; Régnier and Quevauviller, *Bull. sci. pharmacol.*, 43 (1936): 401.

[86] Moore and Roaf, *Proc. Roy. Soc.*, 73 (1904): 382; 77 (1906): 86.

[87] Rider, *J. Pharmacol.*, 39 (1930): 457; *ibid.*, 47 (1933): 255.

[88] Rider, *ibid.*, 40 (1930): 7.

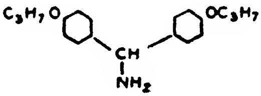
[89] Dietzel and Steeger, *Arch. Pharm.*, 271 (1933): 521.

[89a] Moore, personal communication.

LOCAL ANESTHETICS OTHER THAN AMINOESTERS.

Although most of the local anesthetics in general use are of the aminoester type many other classes of organic compounds exhibit local anesthetic properties in varying degree. The existence of other local anesthetics does not vitiate such conclusions as we have drawn concerning the relation of structure to activity but it does suggest that we have not discovered the fundamental relationship. This fundamental relationship, if it really exists, can only be discovered by the study of an ever increasing number of compounds.

Amines. Simple amines, especially of high molecular weight, have local anesthetic activity. In this series primary amines are more active than secondary, which, in turn, are more active than tertiary. This order is the reverse of that found in the aminoester series. Benzhydrylamine, for example, is very active. When propoxyl groups are substituted on the benzene rings of this amine, a compound,



is obtained which is six times as active and persistent as cocaine.[90] Other alkoxylated benzhydrylamines are potent anesthetics but are generally more toxic than cocaine and rather irritating.[91] Pyridine and quinoline have some anesthetic action[92] and 2-aminopyridine and derivatives are especially active.[93] N-aralkylmorpholines also have anesthetic activity.[93a]

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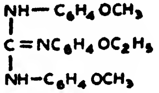
[90] Torres, *Anales soc. espan. fis. quim*, 24 (1926): 82.

[91] Régnier and Sallé, *Bull. sci. pharmacol.*, 33 (1926): 91, 148.

[92] Brunton and Tunicliffe, *J. Physiol.*, 17 (1894): 272; Donath, *Ber.*, 14 (1881): 178, 1769; Stockmann, *J. Physiol.*, 15 (1892): 245; Santesson and Koraen, *Skand. Arch. Physiol.*, 10 (1900): 201.

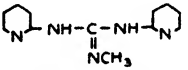
[93] Camps, *Arch. Pharm.*, 240 (1902): 348; Seide, *Ber.*, 57 (1924): 1802.

[93a] Leffler and Volwiler, *J. Am. Chem. Soc.*, 60 (1938): 896.



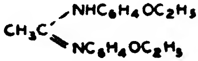
ACOIN

Amidines (including Guanidines). A guanidine called Acoin was introduced a number of years ago.[94] Dipyridyl guanidines have recently been prepared.[95] The best of these, called protocaine, is the methyl guanidine. It can be seen that this compound contains a 2-aminopyridine group.



PROTOCAINE

A large number of simpler amidines have been prepared.[96] Of these, holocaine has been especially useful in ophthalmology but has a high toxicity.[97] The presence of the ethoxyl group is to be noticed.



HOLOCAINE

Amides and Iminoethers. A number of simple aryl ureas have local anesthetic activity.[97a] The amide, niketol,

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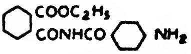
[94] Hesse and Trolldiener, *Therap. Monatsh.* (1899): 36; *Brit. Med. J.*, 1 (1899): 1340; *Cf.* Miescher, *Helv. chim. Acta*, 15 (1932): 163.

[95] Topschiew, *Arch. Pharm.*, 272 (1934): 775; Tzobkalla, *Arch. sci. biol.* (U. S. S. R.), 39 (1935): 239.

[96] Taube, *Chemisches Technologie* (Wagner), 41 (1898): 620, 621; Goldschmidt, German Patents 97,103 (1898), 103,982 (1899); *Chem. Ztg.*, 26 (1902): 743; *J. Chem. Soc.*, 82 (1902): 785; Hill and Rabinowitz, *J. Am. Chem. Soc.*, 48 (1926): 732; Hill and Cox, *ibid.*, 48 (1926): 3214. For amidines in general see Fränkel, 400-402.

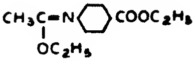
[97] *Zentralbl. prakt. Augenheil.* (1897): 30.

[97a] Wenker, *J. Am. Chem. Soc.*, 60 (1938): 158, 1081.

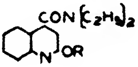


NIKETOL

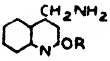
and the iminoether,



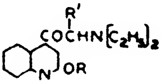
are both local anesthetics. The important member of this class, however, is nupercaine[68] which is both an amide and a cyclic iminoether. This compound has been discussed previously. In the study of the nupercaine type, Wojahn[98] has shown that the simple diethylamides of 2-alkoxycinchoninic acids,



are active local anesthetics, and the ethylene diamine groups in the amide side chain of nupercaine enhances the activity by an increase in the basicity of the molecule. Further, the 2-alkoxy-4-aminomethyl quinolines,



are also active: this is the iminoether portion of the molecule. Interestingly enough, 2-alkoxy-4-quinolyl-α-aminoalkyl ketones,



are vasoconstrictors without anesthetic properties. This is rather surprising since, as will be shown later, many ketones possess local anesthetic activity. Reduced to the lowest term, the 2-alkoxyquinoline group seems to be the activating group in this series.

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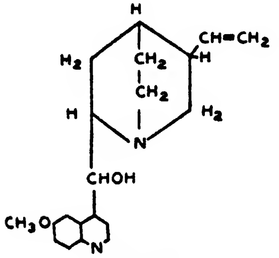
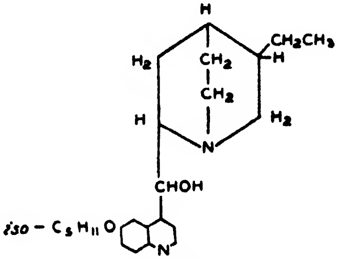
[98] Wojahn, *Arch. Pharm.*, **274** (1936): 83.

While we are discussing cinchoninic acids it should be pointed out that quinine itself has a local anesthetic action and is frequently used, especially in combination with urea or urethane.[99] Numerous derivatives of quinine, chiefly reduction products (hydrocupreins) with various side chains, have been used, and of these eucupin[100] seems to be the best.



QUININE



EUCUPIN

Ketones, Aldehydes, Ethers, Phenols. Many aldehydes are slightly anesthetic but they are too readily oxidized to be of practical importance. Amino aryl-alkyl ketones of the type $C_6H_5COCH_2NC_5H_{10}$ are local anesthetics.[101] The reverse type in which an amino group is substituted in the aromatic portion has been found by Hartung and Munch[102] also to have local anesthetic activity. These are of the type $NH_2C_6H_4COR$, R being alkyl.

As is well known, ethers are general rather than local anesthetics but we have encountered many examples in which the substitution of an ether group, especially in an aromatic nucleus, enhances the activity. Iminoethers have been mentioned. The phenolic group is generally most useful when esterified but it exerts a slight local anesthetic action itself as, for example, in hexyl resorcinol or in phenol itself.

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[99] Schepelmann, *Therap. Gegenw.*, 13 (1911): 545; *Cf.* von Oettingen, The Therapeutic Agents of the Quinoline Group (1933), pp. 150-151.

[100] German Patent 254,702; Morganroth and Ginzberg, *Berlin. klin. Wochschr.*, 49 (1912): 2183; 50 (1913): 343. *Cf.* von Oettingen, *op. cit.*, pp. 197-200.

[101] Mannich and Lammering, *Ber.*, 55 (1922): 3515; Mannich and Curtas, *Arch. Pharm.*, 264 (1926): 750; German Patent 379,950; Blicke and Blake, *J Am. Chem. Soc.*, 52 (1930). 235.

[102] Hartung and Munch, *J. Am. Chem. Soc.*, 51 (1929): 2570.

Alcohols. Macht,[103] interested in the benzyl alcohol portion of the papaverine molecule, found benzyl alcohol to have local anesthetic properties. This led to the investigation of numerous similar alcohols, including β-phenylethyl,[104] salicyl[105] and related alcohols. Interestingly enough, the ethyl ester of salicyl alcohol is without anesthetic properties. Hirschfelder[106] found that primary alcohols are better than secondary which, in turn, are better than tertiary. Among the compounds studied, one of the most active is benzoylcarbinol (or hydroxyacetophenone), $C_6H_5COCH_2OH$, a combination of ketone and alcohol.

RELATION OF PHYSICAL PROPERTIES TO LOCAL ANESTHETIC ACTION

Although we were able to draw some general conclusions with regard to the relation between chemical structure and physiological action in the aminoester series, the relationships were not entirely clear-cut and there were numerous exceptions. When we consider, further, the importance of the salt-forming acid in this series, and finally add the numerous unrelated compounds which exhibit local anesthetic properties, the problem of relating chemical constitution to anesthetic action becomes more difficult. This difficulty is increased because we are not dealing with one form of physiological action alone. In addition to the fundamental local anesthetic activity, which requires that a compound exert a preferential action on sensory as contrasted with motor nerves, the compound must possess properties which enable it to penetrate mucous tissue or the nerve sheath (depending upon the mode of administration), and it must in some way fix itself to the cell to prevent too rapid absorption from the site of application.

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[103] Macht, *J. Pharmacol.*, 11 (1918): 263, 389, 419.

[104] Hjort and Kaufmann, *ibid.*, 15 (1920): 129.

[105] Hirschfelder, Lundholm and Norrgaard, *ibid.*, 15 (1920): 261.

[106] Quigley and Hirschfelder, *ibid.*, 18 (1921): 326.

These requirements and the rather bewildering number of compounds having local anesthetic activity have led to a certain amount of work aimed at relating local anesthetic activity and physical properties. In view of our lack of any very well defined and generally accepted theory of anesthetic action—local or general—it can readily be seen that such attempts have not solved the problem. However, certain suggestive results have been obtained and we shall outline these very briefly. It should be pointed out that, in the end, we may be answering our chemical problem by this approach since chemical constitution must condition the physical properties.

Lipoid Solubility. Most of the physical properties of local anesthetics which have been investigated are carry-overs from the field of general anesthesia. As we have already mentioned, Overton and Meyer[79] presented evidence that general anesthetic properties parallel fat solubility since this would measure the ability of the anesthetic to dissolve in the lipoid portion of the cell. This, it will be recalled, was the basis of the Gros theory,[80] that local anesthetic activity resides in the free-base portion of the aminoester salt molecule. Adams *et al.*[34] determined the oil-water distribution coefficients of a series of alkyl para-aminobenzoates and found, in fact, that in this series local anesthetic efficiency does parallel the distribution coefficient. Rohmann and Scheurle[107] found correlation between lipoid solubility and local anesthetic activity in a series of *p*-hydroxybenzoic ester ethers.

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[107] Rohmann and Scheurle, *Arch. Pharm.*, **274** (1936): 236.

Surface Tension and Adsorption. Traube,[108] feeling that adsorption at the cell interface might be a determining factor in general anesthesia, suggested that surface tension lowering should be a measure of anesthetic action. Warburg developed this into the adsorption theory[109] which it fundamentally is. Adams *et al.*[34] again found that surface tension lowering paralleled local anesthetic efficiency in their series. Miescher,[68b] studying nupercaine and homologs, obtained similar results. Rohmann and Scheurle[110] found a qualitative parallelism in their series. These authors also measured the adsorption on charcoal (liquid-solid rather than liquid-gas interface) but found no correlation. Moore and Roaf,[86] in their studies of local anesthetics and cell proteins, obtained curves indicative of adsorption rather than compound formation. Neither Leffler and Brill[111] nor Gardner and Semb[112] found a relation between local anesthetic activity and surface tension lowering. Régnier did not believe that surface tension lowering was important in cocaine anesthesia.[113]

Coagulation of Proteins. Bancroft[114] revived Claude Bernard's theory[115] that general anesthesia is due to the reversible coagulation of the nerve colloids. Rohmann and Scheurle,[107] testing this theory for local anesthetics by determining the concentration necessary to precipitate an albumose solution, verified it for their series. Rider[87] had earlier pointed out the importance of the protein coagulating power of local anesthetics.

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[108] Traube, *Z. exptl. Path. Therap.*, **2** (1905): 117; *Biochem. Z.*, **98** (1919): 177, 179; *Arch. ges. Physiol.*, **218** (1928): 749; *Biochem. Z.*, **279** (1935): 166.

[109] Warburg, *Arch ges. Physiol.*, **155** (1919): 547; *Biochem. Z.*, **119** (1921): 134.

[110] Rohmann and Scheurle, *Arch. Pharm.*, **274** (1936): 225.

[111] Leffler and Brill, *J. Am. Chem. Soc.*, **55** (1933): 365.

[112] Gardner and Semb, *J. Pharmacol.*, **54** (1935): 309.

[113] Régnier and David, *Bull. sci. pharmacol.*, **32** (1925): 513.

[114] Bancroft and Richter, *J. Phys. Chem.*, **35** (1931): 215.

[115] Bernard, Leçons sur les anesthésiques et sur l'asphyxie, Paris (1875).

Solubility. Rider[88] has suggested that low water solubility of an anesthetic may be desirable in that the high escaping tendency of the anesthetic renders it more readily adsorbed by the cell.

Hydrogen Ion Concentration. We have previously called attention to the increase in local anesthetic potency obtained by alkalinizing local anesthetic solutions. This was attributed to the release of free base which then accumulated in the cell (or at the cell surface) according to the Meyer-Overton theory. Although this theory had apparently received abundant confirmation,[116] Régnier[82, 83, 84, 85] appears to have pointed out inadequacies and has suggested that the ions present are more important than pH in affecting the local anesthetic potency. Hence, the pH may be less important than originally supposed and may be a concomitant rather than a causative factor. It should be pointed out that pH has a very important influence on the stability of local anesthetic solutions.[117] Important in clinical practice is the increased irritation of solutions of low pH.

Molecular Weight. It has been pointed out by Merz[118] that many of the useful local anesthetics have molecular weights of the same magnitude (generally between 250 and 350). Whether this is a coincidental result of the similarity in structure of most local anesthetics or is in itself of importance, is difficult to say. Adams has observed[29] that any increase in molecular weight of the procaine type of compound results in an increase in toxicity as well as in anesthetic activity. In an attempt to decrease the molecular weight Adams has cyclized the ester chain, producing oxazolines,[119] thiazolines,[120] and oxazoles and thiazoles.[121] The toxicity was markedly decreased, but the insolubility of compounds together with the acidity of the salts and their instability in solution has prevented any practical use.

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[116] *Cf.* Gros, *supra* note 80, Copeland and Notton, *supra* note 81, Gardner and Semb, *supra* note 112, Trevan and Boock, *Brit. J. Exptl. Path.*, **8** (1927): 307; Gerlough, *J. Pharmacol.*, **41** (1931): 307.

[117] A large literature exists on the stability of local anesthetic solutions. See for example: Roy, *J. pharm. chim.* (8), **1** (1925): 525; Liot, *Bull. sci. pharmacol.*, **32** (1925): 83; Régnier, Liot and David, *ibid.*, **40** (1933): 271, 353; Régnier and David, *ibid.*, **41** (1934): 468, 547, 595; Dietzel and Steeger, *Arch. Pharm.*, **271** (1933): 521; Dietzel, *Pharm. Zentralhalle*, **75** (1934): 469; Schou and Heim, *Pharm. Acta. Helv.*, **10** (1935): 31; Schou and Abildgaard, *ibid.*, **10** (1935): 38; Dietzel and Kühl, *Arch. Pharm.*, **272** (1934): 721; Lotheisen, *Wien. Klin. Wochschr.*, **42** (1929): 820; Cook, Bambach and Rider, *J. Am. Pharm. Assoc.*, **24** (1935): 269; Cook and Rider, *ibid.*, **26** (1937): 222.

[118] Merz, *Arch. Pharm.*, **270** (1932): 25.

In considering these various physical properties it will be seen that most of them concern means of getting at the cell and fixing the anesthetic there, and probably all are of importance. We are ignorant of just what happens in the cell although there are abundant theories of the mechanism of local anesthetic action. Apparently the theory most in favor at present is the Warburg adsorption theory. According to this theory anesthetics are preferentially attracted to the nerve cells. They are adsorbed to the cell wall, reducing the permeability, and hence the ability to respond to stimuli.[122] Lillie[123] has presented evidence in favor of this theory and has likened the action to that of protective colloids. The lowered ability to respond to stimuli has been investigated in terms of lowered nerve conductivity;[124] indeed, the effect on the electrical properties of nerves has been used in testing local anesthetics.[125]

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[119] Leffler and Adams, *J. Am. Chem. Soc.*, 59 (1937): 2252; Novelli and Adams, *ibid.*, 59 (1937): 2259.

[120] Babcock and Adams, *J. Am. Chem. Soc.*, 59 (1937): 2260.

[121] Friedman, Sparks and Adams, *J. Am. Chem. Soc.*, 59 (1937): 2262.

[122] *Cf.* Schmidt, *Dental Cosmos*, 78 (1936): 717; Winterstein, Die Narkose, 2nd ed., Berlin (1926).

[123] Lillie, *Science*, 37 (1913): 959.

[124] Hughes and King, *Science*, 57 (1923): 590.

It is, however, beyond the scope of this paper to discuss the theories of anesthesia.* Our object has been to indicate briefly the broad outlines of our present knowledge of the chemical constitution of local anesthetics and to suggest, thereby, how much remains to be done.

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[125] Régnier and Quevauviller, *Compt. rend.*, **210** (1935): 912; *Compt. rend. soc. biol.*, **122** (1936): 251; *Bull. sci. pharmacol.*, **43** (1936): 401.

*For a review of the theories of anesthesia, see Henderson.[126]

[126] Henderson, *Physiol. Rev.*, **10** (1930): 171.

GENERAL REFERENCES

A. Books containing information on local anesthetics.

Francis, F., and Fortescue-Brickdale, J. M., The Chemical Basis of Pharmacology, London (1908).

Spiegel, L., Chemical Constitution and Physiological Action, New York (1915).

Barrowcliff, M., and Carr, F. H., Organic Medicinal Chemicals, New York (1920).

May, P., The Chemistry of Synthetic Drugs, London (1921).

Fourneau, E., Organic Medicaments and their Preparation, Philadelphia (1925).

Fränkel, S., Die Arzneimittel-Synthese, Berlin (1927).

Dyson, G. M., The Chemistry of Chemotherapy, London (1928).

Waser, E., Synthese der Organischen Arzneimittel, Stuttgart (1928).

Slotta, K. H., Grundriss der modernen Arzneistoff-Synthese, Stuttgart (1931).

Evers, N., The Chemistery of Drugs, 2nd ed., New York (1933).

Lespagnol, A., Pharmacie Chimique, Paris (1936).

von Oettingen, W. F., The Therapeutic Agents of the Quinoline Group, New York (1933).

von Oettingen, W. F., The Therapeutic Agents of the Pyrrole and Pyridine Group, Ann Arbor (1936).

B. General papers on local anesthetics.

Hirschfelder and Bieter, *Physiol. Rev.*, **12** (1932): 190.

Miescher, *Helv. chim. Acta*, **15** (1932): 163.

Merz, *Arch. Pharm.*, **270** (1932): 125.

Rehn and Killian, *Münch. med. Wochschr.*, 79 (1932): 545.

Killian, *Schmerz Narkose-Anesthesie*, 8 (1935): 153.

LITERATURE CITED

Abramson and Goldberg, *J. Pharmacol*, **62** (1938): 69.

Adams, Rideal, Burnett, Jenkins and Dreger, *J. Am. Chem. Soc.*, **48** (1926): 1758.

Adelson, MacDonald and Pollard, *J. Am. Chem. Soc.*, **57** (1935): 1988.

Andrews and McElvain, *J. Am. Chem. Soc.*, **51** (1929): 887.

Babcock and Adams, *J. Am. Chem. Soc.*, **59** (1937): 2260.

Bachman, *J. Am. Chem. Soc.*, **57** (1935): 2167.

Bailey and McElvain, *J Am. Chem. Soc.*, **52** (1930): 1633, 2007.

Bancroft and Richter, *J. Phys. Chem.*, **35** (1931): 215.

Barnes and Adams, *J. Am. Chem. Soc.*, **49** (1927): 1307.

Barrowcliff, M., and Carr, F. H., Organic Medicinal Chemicals, New York (1920).

Bayer, German Patent 173,631.

Bernard, Leçons sur les anesthésiques et sur Lásphyxie, Paris (1875).

Berret, Thèse, Geneva (1931).

Bjerregaard and Houstin, *Proc. Okla. Acad. Sci.*, **14** (1934): 77.

Blicke and Blake, *J. Am. Chem. Soc.*, **52** (1930): 235; **53** (1931): 1015.

Bodendorf, *Deut. Apoth. Ztg.*, 49 (1934): 1642.

Boese and Major, *J. Am. Chem. Soc.*, **57** (1935): 175.

Bolyard and McElvain, *J. Am. Chem. Soc.*, **51** (1929): 922.

Bonar and Sollman, *J. Pharmacol.*, 18 (1921): 467.

Braun, Die örtliche Betäubung, Leipzig, 6th ed. (English translation of the third edition by Shields, Phila., 1914.)

von Braun and Braunsdorf, *Ber.*, **54** (1921): 2081.

von Braun and Kirschbaum, *Ber.*, **52** (1919): 2011.

von Braun and Müller, *Ber.*, **51** (1918): 235.

Brill, *J. Am. Chem. Soc.*, **47** (1925): 1134; **54** (1932): 2484.

Brill and Bulow, *J. Am. Chem. Soc.*, **55** (1933): 2059.

Brill and Cook, *J. Am. Chem. Soc.*, **55** (1933): 2062.

Brit. Patent 241,767; *Chem. Abst.*, **20** (1926): 3539.

Brunton and Tunicliffe, *J. Physiol.*, **17** (1894): 272.

Burnett, Jenkins, Peet, Dreger and Adams, *J. Am. Chem. Soc.*, **59** (1937): 2248.

Burrows and Reid, *J. Am. Chem. Soc.*, **56** (1934): 1720.

Camps, *Arch. Pharm.*, **240** (1902): 348.

Chadbourne, *Brit. Med. J.* (1892): 402.

Coles and Lott, *J. Am. Chem. Soc.*, **58** (1936): 1989.

Cook, *J. Am. Chem. Soc.*, **59** (1937): 2661; Dissertation, Yale University (1933).

Cook, Bambach and Rider, *J. Am. Pharm. Assoc.*, **24** (1935): 269.

Cook and Hill, to be published.

Cook and Rider, *J. Am. Chem. Soc.*, **58** (1936): 1079; **59** (1937): 1739; *J. Am. Pharm. Assoc.*, **26** (1937): 222.

Cope and McElvain, *J. Am. Chem. Soc.*, **53** (1931): 1587.

Copeland and Notton, *Brit. Med. J.*, **2** (1925): 547.

Dietzel, *Pharm. Zentralhalle*, **75** (1934): 469.

Deitzel and Kühl, *Arch. Pharm.*, **272** (1934): 721.

Dietzel and Steeger, *Arch. Pharm.*, **271** (1933): 521.

Donath, *Ber.*, **14** (1881): 178, 1769.

Dyson, G. M., The Chemistry of Chemotherapy, London (1928).

Ehrlich, *Deut. med. Wochschr.*, **16** (1890): 717.

Einhorn, *Ber.*, **21** (1888): 3029, 3411.

Einhorn, Fiedler, Ladisch and Uhlfelder, *Ann.*, **371** (1909): 142.

Einhorn and Uhlfelder, *Ann.*, **371** (1909): 131.

Ernst, *Münch. med. Wochschr.*, **128** (1931): 9.

Evers, N., The Chemistry of Drugs, 2nd ed., New York (1933).

Fenwick and Gilman, *J. Biol. Chem.* **84** (1929): 605.

Filehne, *Berlin. klin. Wochschr.* (1887): 107.

Fisk and Underhill, *J. Pharmacol.*, **49** (1933): 320.

Fosdick and Hansen, *J. Am. Dental Assoc.*, **21** (1934): 1777; *J. Pharmacol.*, **50** (1934): 323.

Fosdick and Wessinger, *J. Am. Chem. Soc.*, **60** (1938): 1465.

Fourneau, *Compt. rend.*, **138** (1904): 7661; *J. pharm. chim.*, **2** (1910): 56.

Fourneau, Organic Medicaments and their Preparation, Philadelphia (1925).

Francis, F., and Fortescue-Brickdale, J. M., The Chemical Basis of Pharmacology, London (1908).

Fränkel, S., Die Arzneimittel-Synthese, Berlin (1927).

Friedman, Sparks and Adams, *J. Am. Chem. Soc.*, 59 (1937): 2262.

Fromherz, *Arch. exptl. Path. Pharmakol.*, 76 (1914): 257; 158 (1930): 368.

Fussgänger and Schaumann, *Arch. exptl. Path. Pharmakol.*, 160 (1931): 53.

Gardner, Clark and Semb, *J. Am. Chem. Soc.*, 55 (1933): 2999.

Gardner and Hammel, *ibid.*, 58 (1936): 1360.

Gardner and Semb, *J. Pharmacol.*, 54 (1935): 309.

Gardner and Schneider, *J. Am. Chem. Soc.*, 55 (1933): 3823.

Gerlough, *J. Pharmacol.*, 41 (1931): 307.

German Patent 88,270; 90,069; 254,702.

Giesel, *Pharm. Ztg.* (1891): 149.

Gilman and Pickens, *J. Am. Chem. Soc.*, 47 (1925): 245; 50 (1928): 437.

Goldberg and Whitmore, *J. Am. Chem. Soc.*, 59 (1937): 2280.

Goldschmidt, German Patents 97,103 (1898), 103,982 (1899); *Chem. Ztg.*, 26 (1902): 743; *J. Chem. Soc.*, 82 (1902): 785.

Gros, *Arch. exptl. Path. Pharmakol.*, 62 (1910): 380; 63 (1910): 80; 67 (1912): 126.

Hansen and Fosdick, *J. Am. Chem. Soc.*, 55 (1933): 2872.

Hartung and Munch, *J. Am. Chem. Soc.*, 51 (1929): 2570.

Heckel with Adams, *J. Am. Chem. Soc.*, 49 (1927): 1303.

Henderson, *Physiol. Rev.*, 10 (1930): 171.

Hesse and Trolldiener, *Therap. Monatsh.* (1899): 36; *Brit. Med. J.*, 1 (1899): 1340.

Hill and Rabinowitz, *J. Am. Chem. Soc.*, 48 (1926): 732.

Hill and Cox, *ibid.*, 48 (1926): 3214.

Hirschfelder and Bieter, *Physiol. Rev.*, 12 (1932): 190.

Hirschfelder, Lundholm and Norrgaard, *J. Pharmacol.*, 15 (1920): 261.

Hjort and Kaufmann, *J. Pharmacol.*, 15 (1920): 129.

Horne, Cox and Shriner, *J. Am. Chem. Soc.*, 55 (1933): 3435.

Hughes and King, *Science*, 57 (1923): 590.

Impens, *Arch. ges. Physiol.* (*Pflügers*), 110 (1905): 21.

Kamm, *J. Am. Chem. Soc.*, 42 (1920): 1030.

Kamm, Adams and Volwiler, U. S. Patents, 1,358,750 and 1,358,751.

Karjala and McElvain, *J. Am. Chem. Soc.*, 55 (1933): 2966.

Karrer, *Helv. chim. Acta.*, 4 (1921): 92.

Killian, *Schmerz Narkose-Anesthesie*, 8 (1935): 153.

Knoefel, *J. Pharmacol.*, **47** (1933): 69.

Kurvahata, Ochiai and Nukita, *Folia Pharmacol. Japon.*, **7** (1928): 408.

Leffler and Adams, *J. Am. Chem. Soc.*, **59** (1937): 2252.

Leffler and Brill, *J. Am. Chem. Soc.*, **55** (1933): 365.

Leffler and Volwiler, *J. Am. Chem. Soc.*, **60** (1938): 896.

Lespagnol, A., Pharmacie Chimique, Paris (1936).

Liebermann, *Ber.*, **24** (1891): 2336, 2587; **25** (1892): 927.

Lillie, *Science*, **37** (1913): 959.

Liot, *Bull. sci. pharmacol.*, **32** (1935): 83.

Lischer and Jordan, *J. Am. Chem. Soc.*, **59** (1937): 1623.

Lotheisen, *Wien. Klin. Wochschr.*, **42** (1929): 820.

McElvain, *J. Am. Chem. Soc.*, **46** (1924): 1721; **48** (1926): 2179, 2239; **49** (1927): 2835, 3348.

Macht, *J. Pharmacol.*, **11** (1918): 263, 389, 419.

Macht and Burrows, *Am. J. Physiol.*, **113** (1935): 92.

Mannich, Borkowsky and Lin, *Arch. Pharm.*, **275** (1937): 54.

Mannich and Braun, *Ber.*, **53** (1920): 1874.

Mannich and Curtas, *Arch. Pharm.*, **264** (1926): 750; German Patent 379,950.

Mannich and Lammering, *Ber.*, **55** (1922): 3515.

Marvel and Shelton, *J. Am. Chem. Soc.*, **51** (1929): 915.

May, P., The Chemistry of Synthetic Drugs, London (1921).

Merck, *Ber.*, **18** (1885): 2954; **21** (1888): 48.

Merling, *Ber. deut. pharm. Ges.*, **6** (1897): 173.

Merz, *Arch. Pharm.*, **270** (1932): 125.

Meyer, *Arch. exptl. Path. Pharmacol.*, **46** (1901): 338.

Miescher, *Helv. chim. Acta*, **15** (1932): 163; U. S. Patent 1,688,469 (1928).

Moore, personal communication.

Moore and Roaf, *Proc. Roy. Soc.*, **73** (1904): 382; **77** (1906): 86.

Moreno y Maiz, Thèse de Paris (1868).

Morganroth and Ginzberg, *Berlin. klin. Wochschr.*, **49** (1912): 2183; **50** (1913): 343.

Mozans, Along the Andes and Down the Amazon, New York (1911): 206, 207.

Munch, Bioassays, Baltimore (1931): 54.

Niemann, *Ann.*, **140** (1860): 213.

Novelli and Adams, *J. Am. Chem. Soc.*, **59** (1937): 2259.

Novy, *Am. Chem. J.*, 10 (1888): 147.

von Oettingen, *Ann. Med. Hist.*, 5 (1933): 275.

von Oettingen, The Therapeutic Agents of the Quinoline Group, New York (1933).

von Oettingen, The Therapeutic Agents of the Pyrrole and Pyridine Group, Ann Arbor (1936).

Osterberg and Kendall, *J. Am. Chem. Soc.*, 43 (1921): 1370.

Overton, Studien über die Narkose, Jena (1901).

Phatak and Emerson, *J. Pharmacol.*, 58 (1936): 174.

Pyman, *J. Chem. Soc.*, 93 (1908): 1793, 1802; 111 (1917): 167, 1119.

Quigley and Hirschfelder, *J. Pharmacol.*, 18 (1921): 326.

Régnier, *Bull. sci. pharmacol.*, 31 (1924): 513; 32 (1925): 271; French Patent 789,156.

Régnier and David, *Bull. sci. pharmacol.*, 32 (1925): 513; 41 (1934): 321, 468, 547, 595; *Compt. rend.*, 200 (1935): 1428; *Anesthésie et Analgésie*, 1 (1935): 285; *J. pharm. chim.*, 22 (1935): 16.

Régnier, Delange and David, *Compt. rend.*, 202 (1936): 591.

Régnier, Lambin and Szollosy, *Compt. rend. soc. biol.*, 122 (1936): 759.

Régnier, Liot and David, *Bull. sci. pharmacol.*, 40 (1933): 271, 353.

Régnier and Quevauviller, *Compt. rend.*, 210 (1935): 912; *Compt. rend. soc. biol.*, 122 (1936): 251; *Bull. sci. pharmacol.*, 43 (1936): 401.

Régnier and Sallé, *Bull. sci. pharmacol.*, 33 (1926): 91, 148.

Rehn and Killian, *Münch. med. Wochschr.*, 79 (1932): 545.

Rider, *J. Am. Chem. Soc.*, 52 (1930): 2115, 2583; *J. Pharmacol.*, 39 (1930): 457; 40 (1930): 7; 47 (1933): 255.

Rider and Cook, *J. Am. Chem. Soc.*, 59 (1937): 1741; *J. Pharmacol.*, 64 (1938):1.

Ritsert, *Pharm. Ztg.*, 70 (1925): 1006.

Robinson and Hill, Organic Chem. Symposium, Columbus, Ohio (Dec., 1927).

Rohmann and Scheurle, *Arch. Pharm.*, 274 (1936): 225, 236.

Roy, *J. pharm. chim.* (8), 1 (1925): 525.

Sandborn and Marvel, *J. Am. Chem. Soc.*, 50 (1928): 563.

Santesson and Koraen, *Skand. Arch. Physiol.*, 10 (1900): 201.

Schepelmann, *Therap. Gegnw.*, 13 (1911): 545.

Schmidt, *Dental Cosmos*, 78 (1936): 717.

Schmitz and Loevenhart, *J. Pharmacol.*, 24 (1924): 159.

Schou and Heim, *Pharm. Acta. Helv.*, 10 (1935): 31.

Schou and Abildgaard, *ibid.*, 10 (1935): 38.

Schulemann, *Klin. Wochschr.*, 3 (1924): 676.

Seide, *Ber.*, 57 (1924): 1802.

Shriner and Keyser, *J. Am. Chem. Soc.*, 60 (1938): 286.

Singer, A Short History of Medicine, New York (1928): 26.

Smith, Dissertation, Yale University (1929).

Smith and Hatcher, *J. Pharmacol.*, 9 (1917): 231.

Slotta, K. H., Grundriss der modernen Arzneistoff-Synthese, Stuttgart (1931).

Spiegel, L., Chemical Constitution and Physiological Action, New York (1915).

Stockmann, *J. Physiol.*, 15 (1892): 245.

Taube, *Chemisches Technologie* (Wagner), 41 (1898): 620, 621.

Thayer and McElvain, *J. Am. Chem. Soc.*, 49 (1927): 2862; 50 (1928): 3348.

Thorp, U. S. Patent 1,193,634.

Topschiew, *Arch. Pharm.*, 272 (1934): 775.

Torres, *Anales soc. espan. fis. quim*, 24 (1926): 82.

Traube, *Z. exptl. Path. Therap.*, 2 (1905): 117; *Biochem. Z.*, 98 (1919): 177, 179; *Arch. ges. Physiol.*, 218 (1928): 749; *Biochem. Z.*, 279 (1935): 166.

Trevan and Boock, *Brit. J. Exptl. Path.*, 8 (1927): 307.

Tzobkalla, *Arch. sci. biol.* (U. S. S. R.), 39 (1935): 239.

U. S. Patents 812,554; 1,474,576.

Vinci, *Arch. path. Anat.* (*Virchow's*), 149 (1897): 201.

Vliet and Adams, *J. Am. Chem. Soc.*, 48 (1926): 2158.

Volwiler, *Science*, 53 (1921): 145.

Volwiler and Adams, U. S. Patent 1,476,934.

Walter, *J. Am. Chem. Soc.*, 60 (1938): 2467.

Warburg, *Arch. ges. Physiol.*, 155 (1919): 547; *Biochem. Z.*, 119 (1921): 134.

Waser, E., Synthese der Organischen Arzneimittel, Stuttgart (1928).

Wenker, *J. Am. Chem. Soc.*, 60 (1938): 158, 1081.

Wildman and Thorp, U. S. Patent 1,193,649.

Willstätter, *Ber.*, 29 (1896): 1575, 2216.

Willstätter, Wolfes and Mäder, *Ann.*, 434 (1923): 11.

Winterstein, Die Narkose, 2nd ed., Berlin (1926).

Wojahn, *Arch. Pharm.*, 269 (1931): 422; 274 (1936): 83.

Zentralbl. prakt. Augenheil. (1897): 30.

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June 15, 1938.

THE RELATION OF PIGMENT PRODUCTION TO THE SIZE OF SEEDS

BY WILLIAM A. BECK, S.M.* AND
SISTER M. PETRONELLA SCHROEDER, C.PP.S.

In a series of experiments on the relation of the development of plant pigments to the quantity of light received,[1] certain irregularities were observed. The amount of pigment obtained when the exposure to light was 70 hours or more, was less per average hourly yield than that of plants exposed for shorter periods. These results also contradicted the findings of Beck.[2] After analyzing all conditions it was thought that this might be due to the fact that the seeds used in the later experiments (with the longer periods of exposure to light) were somewhat smaller than those used in the earlier ones. This happened quite naturally since the smaller seeds settled to the bottom of the mass.

The present work was carried out to determine whether a relationship exists between the size of the seed and the pigment produced in the seedling. It would have been desirable to use seeds from the same stock employed in the previous experiments in which the seeds were from the same flowers but, unfortunately, the supply was exhausted. The seeds actually employed were of the same species as those in the series of experiments mentioned above and were of the same age. Three groups of seeds were selected according to their relative size and were labeled *large*, *medium* and *small*. The weight of the seeds and the viability were determined for each

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*Lecturing Professor, University of Dayton, Dayton, Ohio.

[1] Beck, W. A., Redman, R., Schroeder, Sr. M. Petronella, C.PP.S., THESE STUDIES, 1 (1937): 245.

[2] Beck, W. A., THESE STUDIES, 1 (1937): 218.

TABLE I

The Pigment Yield of Seedlings Grown from Large, Medium and Small Seeds, Exposed in the Chamber for 40 Hours.

Size of Seeds	Weight of Seeds in g.	Wet Weight of cotyledons in g.	Weight of Pulp in g.	Per cent Viable	Chlorophyll in mg.	Xanthophyll in mg.	Carotene in mg.	$\frac{\text{Chlorophyll}}{\text{Seed Weight}}$	$\frac{\text{Xanthophyll}}{\text{Seed Weight}}$	$\frac{\text{Carotene}}{\text{Seed Weight}}$
Large	14.93	27.4	2.614	95	32.08	2.876	1.284	2.2	0.19	0.08
Medium	13.36	34.5	2.506	95	31.70	2.860	0.757	2.3	0.21	0.05
Small	6.99	15.5	0.745	81	19.66	1.810	0.566	2.8	0.25	0.08

The values given are per 100 seeds.

group. The seeds were sprouted in a light-proof chamber under the usual conditions (temperature 25° C. and 95 to 100 per cent relative humidity). When the seedlings were 90 hours old they were placed in the tank and exposed to light for 40 hours. The exposure conditions were the same for the three groups of seedlings. After exposure the extraction and measurement of chlorophyll, xanthophyll, and carotene were carried out by the usual methods of Schertz[3] and Beck.[4] The wet weight of the cotyledons was determined before the extraction of pigment was begun and the dry weight after the extraction was completed. The results are given in Table I.

The dry weight of the seeds, the wet weight of the cotyledons, the weight of the pulp after the extraction of the pigments, and the vitality test of the seeds are included in the table since a knowledge of these factors throws light on the question of pigment production. Comparisons of the different yields of pigments are given graphically in Fig. 1.

DISCUSSION AND CONCLUSIONS

The weight of the seeds, the weight of the pulp of the cotyledons, and the viability of the seeds are roughly proportional to the relative size of the seeds. The wet weights of the cotyledons are also proportional to the size of the seeds except in the case of the large seeds. The seedlings produced by the large seeds probably absorbed less water than normally during the time of sprouting because the relative humidity was slightly lower than usual. Apparently the development of solid matter and pigment was not materially affected by the unusual condition.

It is probable that the mass of the seeds is proportional to the future capacity of the cotyledons which they produce.

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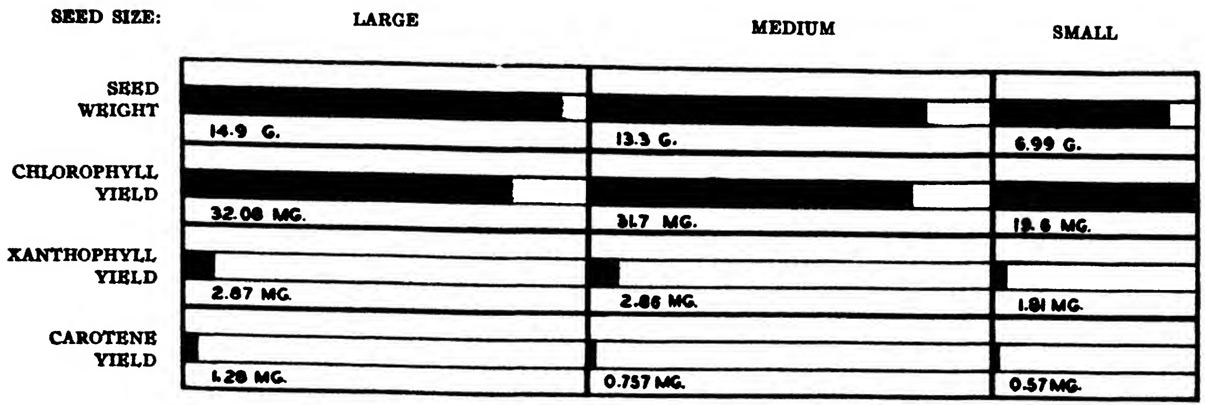
[3] Schertz, F. M., *Plant Physiol.*, 3 (1928): 211.

[4] Beck, W. A., *Science*, 85 (1937): 368.

FIG. 1—*Graphs Showing the Relation Between Weight of the Seeds (in Grams) and the Pigment Developed in Corresponding Seedlings (in Milligrams)*



In other words, small cotyledons emerging from small seeds will not grow quickly enough to produce cotyledons equal in size and capacity to those of seedlings sprouting from larger seeds. From other experiments,[1] it is apparent that the amount of chlorophyll, xanthophyll and carotene which can be produced ultimately in the cotyledons is limited to the capacity of the cotyledons.

The chlorophyll production for the three groups of seeds is in harmony with the seed weights. The large and medium sized, which are about equal in weight (14.9 g. and 13.4 g.), yield about the same amount of chlorophyll (32.1 mg. and 31.7 mg.), and the smaller seeds, which are about half the weight of the others (7.0 g.), yield roughly but half the amount of the pigment (19.7 mg.). The production of the other pigments is similarly proportional to the seed size. It is therefore evident that the amount of pigment produced in the seedlings is roughly proportional to the mass of the seeds as expressed by their respective weights.

It may therefore be concluded that the pigment developed was proportional to the amount of matter present in the seeds.

SUMMARY

1. These experiments indicate an actual relation between the size of seeds and the pigment yielded by the cotyledons after exposure to light for a period of 40 hours.

2. The seedlings produced by the largest seeds yielded the greatest amount of pigment; those produced by the smallest seeds yielded the least. The amount of each pigment was roughly proportional to the mass of the seed.

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CELL ENLARGEMENT IN THE HYPOCOTYL OF HELIANTHUS ANNUUS

By WILLIAM A. BECK,* KLAUS SCHOCKEN AND SISTER MARY WINIFRED DONNELLY, R.S.M.

INTRODUCTION

In these laboratories we are interested in the effects produced by growth promoters. Our group is particularly interested in the effects of the auxins and heteroauxin. *Helianthus annuus* is a favorable subject for experimentation and was chosen as test plant. In order that the effect of growth-promoting substances may be more clearly discerned in cells the normal conditions of the cells in the hypocotyl should be well established. We, therefore, made a preliminary study of the cell enlargement in the hypocotyl.

METHOD

All the seeds (*Helianthus annuus*) employed were from plants grown by us from selected large seeds, taken from the same stock. The seedlings were raised in a dark chamber under controlled conditions. The temperature of the chamber was kept between 80° and 90° F., the relative humidity between 90 per cent and 100 per cent. All plants were 90 hours old when the determinations were made. The epidermal and cortical cells were studied. The sections were taken

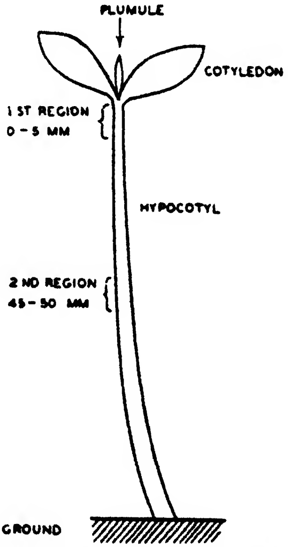

DIAGRAM OF THE SEEDLING OF HELIANTHUS, SHOWING THE REGIONS IN WHICH THE SECTIONS WERE MADE.

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TABLE I

The Enlargement of Epidermal Cells in the Hypocotyl of Helianthus Annuus

	Method Employed	Number of Cells Measured	Location below the Plumule in mm.	Average Area of the Cells in μ^2
Experiment 1	Micrometer Eyepiece	20	0 to 5	761
		20	45 to 50	3455
Experiment 2	Camera Lucida	20	0 to 5	573
		20	45 to 50	3040
Experiment 3	Camera Lucida	20	0 to 5	366
		20	45 to 50	3541
Experiment 4	Micrometer Eyepiece	20	0 to 5	421
		20	45 to 50	3451

from two different regions of the hypocotyl. The first region from 0 to 5 mm. below the plumule contains small young cells, the second region between 45 and 50 mm. contains older and more mature cells. The sections were immediately placed in paraffin oil. This practice is recommended by Ursprung and Blum.[1] According to the experience of Schmidt, Heller and Schilling the oil does not penetrate into the cell and does not exercise any apparent chemical effect upon the contents of the cell. This agrees with the experience of Ursprung and Blum as well as ours. The oil prevents drying of the section by evaporation on the surface.

The sections could not be mounted in water for observation under the microscope since it would enter the cell due to the suction tension of the cell and would change the size in response to the hydrostatic pressure produced by the influx of water.

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[1] Ursprung, A., and Blum, G., *Jahrb. wiss. Botan.*, 63 (1924): 65.

We employed the increment in the area of the cell as an indicator of cell enlargement, following the practice of Ursprung and Blum.[2] The area was measured by two different methods. In the first, an approximation of the area was obtained with the eyepiece micrometer. In the second, the cells were carefully sketched with the aid of the Abbe camera lucida. The magnification was determined with the stage micrometer and the area of the drawing was accurately determined with a planimeter. The arbitrary values obtained from the drawings could readily be translated into absolute values since the magnification was determined. The values were recorded in the tables in μ^2.

DISCUSSION AND CONCLUSION

Epidermal Cells. To obtain the average size of the cells given in the tables 20 cells were employed in each case. The cells employed in Experiments 3 and 4 were identical. In the one case the determination was made with the eyepiece micrometer, in the other with the camera lucida. In making estimations with the eyepiece micrometer, indeterminate errors would be greater in smaller cells than in larger ones, because the approximations made for those portions of irregular cells that did not fit into strictly geometrical forms would produce a greater fractional error in a small total area than in a large total area. In Experiments 3 and 4 the area of the small cells (in the first region) as determined with the camera lucida was $366\mu^2$, but as determined with the eyepiece micrometer was $421\mu^2$, a difference of $55\mu^2$. Considering the method by the camera lucida more accurate than the method by the eyepiece micrometer, we base the error on the

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[2] Ursprung, A., and Blum, G., *Ber. deut. botan. Ges.*, 34 (1916): 525.

TABLE II

The Enlargement of Cortical Cells in the Hypocotyl of Helianthus Annuus

	Method Employed	Number of Cells Measured	Location below the Plumule in mm.	Average Area of the Cells in μ^2
Experiment 5	Camera Lucida	20	0 to 5	2380
		20	45 to 50	14340

values obtained by the camera lucida method. The fractional error then was 55/366 or 0.15, i.e., a 15 per cent error. The per cent error is greater in the smaller cells than in the larger cells, as was expected.

The areas obtained for the small cells in Experiments 1, 2 and 3 are not nearly the same, $761\mu^2$, $573\mu^2$ and $366\mu^2$. It must be remembered that the plants employed were not the same and that no effort was made to distinguish between sections that were made in the uppermost portion of the first region and the lowermost portion of the same region. It is very probable that the cells in the uppermost portion are smaller than in the lowermost portion. There can be no doubt that the order of magnitude is the same. In the larger cells there is a very close agreement showing that in these mature cells no considerable variations in areas of cells occur, whether they are chosen from the uppermost portion or the lowermost portion of the second region.

Choosing Experiment 3 as an example, the difference between the average of the mature cells and the younger cells was $3175\mu^2$ ($3451 - 366$). This represents an enlargement of 9.7 times.

Cortical Cells. Similar studies were made for the cortical cells and the results are recorded in Table II. The younger cells had an average area of $2380\mu^2$, the more mature cells an average of $14{,}340\mu^2$. There was an enlargement of 6 times.

It is evident at once that these cells are considerably larger than the epidermal cells. In Experiment 3 of Table I the average of the younger cells was $366\mu^2$ while the average area of the younger cortical cells was $2380\mu^2$. The average area of the older epidermal cells in Experiment 3 of Table I was $3541\mu^2$, while the average area of the older cortical cells was $14{,}340\mu^2$. These results are in agreement with the effects observed when the hypocotyl is cut lengthwise and placed in water. The cortical cells absorb much more water and enlarge much more considerably under the hydrostatic pressure than do the epidermal cells. While the function of the epidermal cells is more protective, that of the cortical cells is more dynamic. When the hypocotyl was split lengthwise and placed in water the two halves curved in such a manner that the epidermis lay on the concave side and the cortical on the convex side. This effect clearly shows that the suction tension[3] of the cortical cells is greatly increased when the pressure of the epidermal tissue is eliminated, since the two tissues were in equilibrium before sectioning. In the normal state the combined hydrostatic pressure of the epidermal cells produces a physical pressure upon the tissues lying within, reducing their suction tension. This lateral pressure would also have the effect of causing the cortical cells to enlarge more longitudinally than laterally.

SUMMARY

A study of the enlargement of cells in the hypocotyl of *Helianthus annuus* was made. Two methods for determining the areas of the cells were employed. In the first, the eyepiece micrometer was used. In the second, drawings were made with the camera lucida and the areas were determined with the planimeter. The second method was considered more accurate.

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[3] Beck, W., A., *Plant Physiol.*, 3 (1928): 413; Ursprung, A., *Plant Physiol.*, 10 (1935): 115.

The mature epidermal cells in the region between 45 to 50 mm. below the plumule were almost ten times as large as the younger cells in the region between 0 and 5 mm. below the plumule.

The cortical cells in the same relative regions show a six-fold enlargement. The cortical cells are considerably larger than the epidermal cells, which is in agreement with the notion that their function is more dynamic than that of the epidermal cells. The pressure produced by the epidermal cells reduces the suction tension of the tissues lying within, and tends to reduce lateral enlargement.

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EFFECT OF RESPIRATORY AND GROWTH-STIMULATING FACTORS FROM YEAST AND MALT COMBINGS ON BACTERIAL GROWTH

BY JOHN R. LOOFBOUROW AND
SISTER MARY NORBERT MORGAN, R.S.M.

In an earlier paper[1] we published preliminary investigations indicating that certain partially-purified "bios" preparations from yeast and malt combings have specific action on the growth of various strains of bacteria, stimulating the growth of some and inhibiting that of others. These studies were originally undertaken to see whether certain metabolic stimulating factors, or "intercellular hormones"* isolated in these laboratories from various types of cells contained identical or different active principles. For instance, it had been demonstrated that yeast[2] and animal tissues[3] injured by ultraviolet radiation produce metabolic stimulating factors as a result of such injury. Investigation of these factors had been paralleled by the study of materials stimulating respiration, fermentation and proliferation obtained from normal cells.[4] In preparing the latter materials, the earlier stages of extraction and purification had followed the methods employed by Narayanan[5] and Lucas[6] in the preparation of "bios".

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[1] Loofbourow, J. R., Norris, R. J., and Morgan, Sr. M. Norbert, R.S.M., THESE STUDIES, 1 (1937): 193.

*There has been a tendency to give specific names, such as "bios", "pantothenic acid", "traumatin", etc. to unpurified growth factors from various sources. We feel that this is rather premature, as the eventual identification of the chemical structure of the active principles will lead to their being definitely designated by chemical names. It has seemed to us more reasonable, up to the present, to employ a group name for what appears to be a variety of factors controlling cell metabolism. Since the factors in which we are particularly interested seem to be natural products of cells which control the metabolic states of other cells in the cell community, we have regarded these factors as being hormone-like, and to distinguish them from the usual hormones, in the sense of products of cells which are carried through the blood stream to act upon cells in a remote region, we have called them "intercellular hormones". Recently, Professor Cornelius Jansen of our laboratories has suggested the name "biodynes" for these metabolic-controlling factors.

[2] Fardon, J. C., Carroll, Sr. M. Jordan, O.P., and Ruddy, Sr. M. Veronita, O.P., THESE STUDIES, 1 (1937): 17; Fardon, J. C., and Ruddy, Sr. M. Veronita, O.P., *ibid.*, 1 (1937): 41; Fardon, J. C., Norris, R. J., Loofbourow, J. R., and

The investigations on bacteria have, however, taken on new significance in the light of more recent inquiries. Modification of the fractionation procedures employed in preparing the factors, and parallel studies on yeast of the growth, fermentation and respiration stimulation of the fractions so prepared have indicated that there are active principles primarily responsible for stimulating growth, others responsible for stimulating respiration, and still others responsible for stimulating fermentation.[7, 8] Indications have also been obtained that there are a multiplicity of respiratory factors obtainable from different types of cells, each of which exhibits a considerable degree of specificity in its action on various types of cells and tissues.[8]

Other (as yet unpublished) studies by our colleagues, Gallaway and Gallaway, have indicated that a bactericide, phenyl

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Ruddy, Sr. M. Veronita, O.P., *Nature,* **139** (1937): 589; Sperti, G. S., Loofbourow, J. R., and Dwyer, Sr. Cecelia Marie, S.C., *Nature,* **140** (1937): 643; These Studies, **1** (1937): 163; Loofbourow, J. R., Dwyer, Sr. Cecelia Marie, S.C., and Morgan, Sr. M. Norbert, R.S.M., This Issue, p. 137.

[3] Sperti, G. S., Loofbourow, J. R., and Lane, Sr. M. Michaella, S.C., *Science,* **86** (1937): 611; Loofbourow, J. R., Cueto, A. A., and Lane, Sr. M. Michaella, S.C., *Arch. exptl. Zellforsch.,* in publication.

[4] Norris, R. J., and Ruddy, Sr. M. Veronita, O.P., These Studies, **1** (1937): 53; Norris, R. J., and Kreke, C. W., *ibid.,* **1** (1937): 137.

[5] Narayanan, B. T., *Biochem. J.* **24** (1930): 6.

[6] Lucas, G. H. W., *J. Phys. Chem.,* **28** (1924): 1180.

[7] Norris, R. J., and Hart, Sr. M. Jane, O.P., These Studies, **1** (1937): 65.

[8] Cook, E. S., and Kreke, C. W., This Issue, p. 47; Cook, E. S., Kreke, C. W., and Nutini, L. G., *ibid.,* p. 23.

mercuric nitrate, depresses epithelial tissue respiration. It occurred to us that if antiseptics and bactericides generally depress the respiration of tissue cells, this might be partially responsible for their injurious effect on tissues, and if respiratory-stimulating factors which have little effect in stimulating bacterial growth could be used in combination with them, tissue injury due to respiration depression might be reduced without interfering with the bactericidal action. As a first step in investigating this problem, we have endeavored to see if, among the factors we were studying on bacterial growth, those which had a high ratio of respiration to growth stimulation on yeast had correspondingly little effect on the growth of bacteria. The preliminary results have indicated this to be the case, which is encouraging to the undertaking of further studies on more highly purified preparations. Meanwhile, Gallaway and Gallaway (unpublished investigations) have extended their studies to several other antiseptics, all of which depressed tissue respiration. This is at least indicative that the depressing effect may be quite general for bactericides and antiseptics.

REVIEW OF THE LITERATURE

Before proceeding to the experimental discussion, it seems advisable to consider certain important contributions to the literature on bacterial growth factors.

Interest in growth factors for bacteria finds its origin in the inadequacy of purely synthetic media for supporting the growth of micro-organisms. Pasteur[9] used yeast ash in his synthetic fermentation medium. Wildiers[10] noted that the addition of filtrates from yeast suspensions increased the proliferation rate of *Saccharomyces cerevisiae* in a medium of sugar, salts and water. He called the active principle of the filtrate "bios". In later years there was much controversy over the existence of "bios". Tanner's excellent review[11] of the literature up to 1924 came to rather negative conclusions.

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[9] Pasteur, L., Studies on Fermentation, Paris, (1876) p. 331.

[10] Wildiers, E., *La Cellule*, 18 (1901): 313.

When it was found that vitamin B_1 had marked proliferation-promoting action on micro-organisms, interest in the "bios" question received a new impetus. Some investigators tested vitamin B_1 on micro-organisms, others renewed efforts at the purification of "bios" from yeast, and still others endeavored to prepare proliferation promoters from other sources. The chemical aspects of recent investigations of the "bios" question have been reviewed by Norris and Kreke.[12]

In most of this work, the biologic testing was done upon yeast. A few investigators concerned themselves with proliferation promoters for bacteria.

Knight[13] concluded that one of the active constituents of a proliferation-promoting preparation for *Staphylococcus* was vitamin B_1 and the other (as evidenced by Holiday's[14] spectrographic results) nicotinic acid.

Following the lead of Schopfer, and Schopfer and Jung,[15] who found that vitamin B_1 stimulates the growth of *Phycomyces* and later were able to replace the vitamin by its pyrimidine and thiazole components, Sinclair[16] tested the pyrimidine (together with certain substitution products thereof) and thiazole components of vitamin B_1 on *Staphylococci* and observed that they were active together but not alone. Tatum,

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[11] Tanner, F. W., *Chem. Rev.* 1 (1925): 397.

[12] Norris, R. J., and Kreke, C. W., THESE STUDIES, 1 (1937): 137.

[13] Knight, B. C. J. G., *Biochem. J.*, 31 (1937): 731; *Nature*, 139 (1937): 628; *J. Soc. Chem. Ind.*, 56 (1937): 445.

[14] Holiday, E. R., *Biochem. J.* 31 (1937): 1299.

[15] Schopfer, W. H., *Arch. Mikrobiol*, 5 (1934): 502, 511; 6 (1935): 139, 196, 334; *Compt. rend.*, 200 (1935): 1965; *Ber. deut. botan. Ges.*, 54 (1936): 559; *Z. Vitaminforsch.*, 4 (1937): 187; Schopfer, W. H., and Jung, A., *Compt. rend.*, 204 (1937): 1500; *Arch., Mikrobiol*, 6 (1935): 345.

[16] Sinclair, H. M., *Nature*, 140 (1937): 361.

Wood and Peterson[17] found that, in addition to vitamin B_1, extracts of potato, orange juice and yeast stimulate the growth of propionic acid bacteria. A water-soluble extract from yeast had a similar effect on lactic acid bacteria. Richardson[18] found uracil essential to the anaerobic growth of *Staphylococcus*, but not to its aerobic growth. Koser, Saunders and associates[19] prepared a growth factor for pathogenic bacteria from veal infusion and various similar sources. Pulkki[20] obtained a product from boiled yeast which stimulated the growth of *Bacillus mucoides*. Elder[21] found that a "bios" concentrate from tomato juice stimulated the growth of various micro-organisms. Lwoff and Lwoff[22] demonstrated that a "vitamin" factor essential for the growth of *Bacillus influenzae* is cozymase.

Since the methods of extraction employed in preparing many of the crude materials discussed above were similar to those used in the early stages of preparation of the growth and respiratory stimulating factors we employed, it is not surprising that we have found some of our concentrates to stimulate the growth of certain strains of bacteria.

EXPERIMENTAL

Preparation of the "Bios" Fractions. Six preparations were employed in the present investigations: three "bios" extracts from malt combings, one "bios" preparation from yeast, cell-free filtrates from suspensions of yeast injured by ultraviolet irradiation, and cell-free filtrates from control yeast suspensions not exposed to ultraviolet.

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[17] Tatum, E. L., Wood, H. G., and Peterson, W. H., *Biochem. J.*, **30** (1936): 1898; *J. Bact.*, **32** (1936): 167; **33** (1937): 227; Tatum, E. L., Peterson, W. H., and Fred, E. B., *J. Bact.*, **32** (1936): 157; Snell, E. E., Tatum, E. L., and Peterson, W. H., *J. Bact.*, **33** (1937): 207.

[18] Richardson, G. H., *Biochem. J.* **30** (1936): 2184.

[19] Koser, S. A., Saunders, F., Finkle, I. J., and Spoelstra, R. C., *J. Infectious Diseases*, **58** (1936): 121, 127; Koser, S. A., and Saunders, F., *J. Bact.*, **29** (1935): 17.

[20] Pulkki, L. H., *Ann. Acad. Sci. Fennicae*, A-41, No. 1 (1935): 141.

[21] Elder, M. L., *Trans. Roy. Soc. Can.*, **33** (1936): 89

[22] Lwoff, A., and Lwoff, M., *Proc. Roy. Soc. (London)*, B-122 (1937): 352.

The yeast "bios" was prepared by the method of Narayanan[5] and corresponded to his 80 per cent alcohol extract of the lead acetate filtrate.

The fractionation of "bios" from malt combings was carried out by Norris and Kreke,[12] following the method of Lucas.[6] Their *Sample 8* corresponded to Lucas' filtrate from the lead acetate-ammonia precipitation, and their *Samples 14-1* and *14-2* to Lucas' acetone precipitate.

Preparation of Yeast Wound Hormone. The cell-free filtrates from yeast injured by irradiation with ultraviolet were prepared from Fleischmann's bakers' yeast, 3.5 g., taken from the center of the cake, being emulsified in 200.0 cc. of sterile 0.85 per cent sodium chloride solution. This was irradiated in a quartz tube, with constant stirring, for 55 minutes at a distance of 25 cm. from a Burdick quartz mercury arc operated at 65 volts. In a few cases, 40 g. of yeast were suspended in the same amount of sterile saline and irradiated for 3.5 hours at the same distance. The times of irradiation were chosen to result in injury of approximately 90 per cent of the cells, as determined by methylene blue tests. Control suspensions of the same concentrations, in glass containers covered with black paper, were placed at the same distance from the arc as those receiving ultraviolet irradiation. The temperature ranged from 35° to 37° C.

Berkefeld filters (N type) were used to separate the intercellular fluid from the yeast cells. Some of the filtrates were preserved as prepared. Others were taken to dryness in a hot air oven, and taken up in solution in the concentration desired.

Growth Assays. The growth activity of the preparations was assayed on yeast grown in Reader's medium in rocker

TABLE I

Preliminary Experiments on Stimulation of Growth of Bacteria by Sample 14-1, a "Bios" Extract from Malt Combings

Organism	Hours of Incubation	Average Counts		Results
		Controls	9.6 mg. "Bios"/cc.	
Streptococcus haemolyticus	48	182,412	162,144	Inhibition
Staphylococcus aureus	24	199,302	530,346	Marked stimulation
Diplococcus pneumoniae, Type III	24	111,608	111,608	No stimulation
Alkaligenes faecalis	24	1,418,760	1,486,320	No stimulation
Aerobacter aerogenes	24	810,720	743,160	Inhibition
Bacillus pyocyaneus	24	6,080,401	6,137,960	No stimulation

The assay of the "bios" extract from malt combings *Sample 14-1* on yeast was:
10.6 growth units/mg.
1.5 respiration units/mg.

tubes according to techniques previously described.[2] The respiration assays were made by Norris and Kreke, using methods described in their paper.[12]

For the studies on bacteria, six strains were chosen. These were grown in nutrient broth (Difco), except that Hartley broth was used in some instances for culturing *Streptococcus haemolyticus* and *Diplococcus pneumoniae, Type III*. The bacteria were inoculated in quantities of 0.1 cc. from suspensions which were adjusted in all experiments to approximately the same density. The total volume in each growth tube was 7 cc., of which 5 cc. was nutrient medium. The growth preparations were added in amounts from 0.1 cc. to 2.0 cc., and the remaining volume made up with 0.85 per cent salt solution. Salt solution alone was added to the controls. The tubes were incubated 24 hours at 37.5 ° C.

Four methods were used in estimating growth in the suspensions: (1) counts were made from stained smears on a ruled slide to which a drop of suspension was applied with a standard loop, (2) equal volumes of the suspensions were plated on nutrient agar (Difco), (3) 5.0 cc. portions of the suspensions were pipetted into Hopkins' vaccine tubes and centrifuged for 20 minutes at a standard speed, and (4) the optical densities of the suspensions were measured with a photoelectric photodensitometer.[23]

Preliminary Results. In Table I are summarized the results of a preliminary series of experiments in which *Sample 14-1* from malt combings was tested on five strains of bacteria and the growth after 24 hours was determined by counting smears. Stimulation was obtained only with *Staphylococcus aureus.* With *Aerobacter aerogenes* there was some indication of inhibition.

It was clear from these experiments that the growth factor (or factors) present in the preparation was specifically active on particular strains of bacteria.

In these preliminary experiments, a single concentration of the growth-promoting preparation (9.6 mg. per cc. of suspension) was used. In the later experiments, serial dilutions of the growth factors, usually covering a range of 20 to 1, were always employed. This gave a better indication of the consistency of the results.

These preliminary experiments were considered only as giving a qualitative indication of the type of results to be expected.

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[23] Beck, W. A., *Science,* 85 (1937): 368.

FIG. 1—*Effect of Concentration of Growth Factors on Growth of Bacteria. Values from Individual Experiments—Not Averages.*

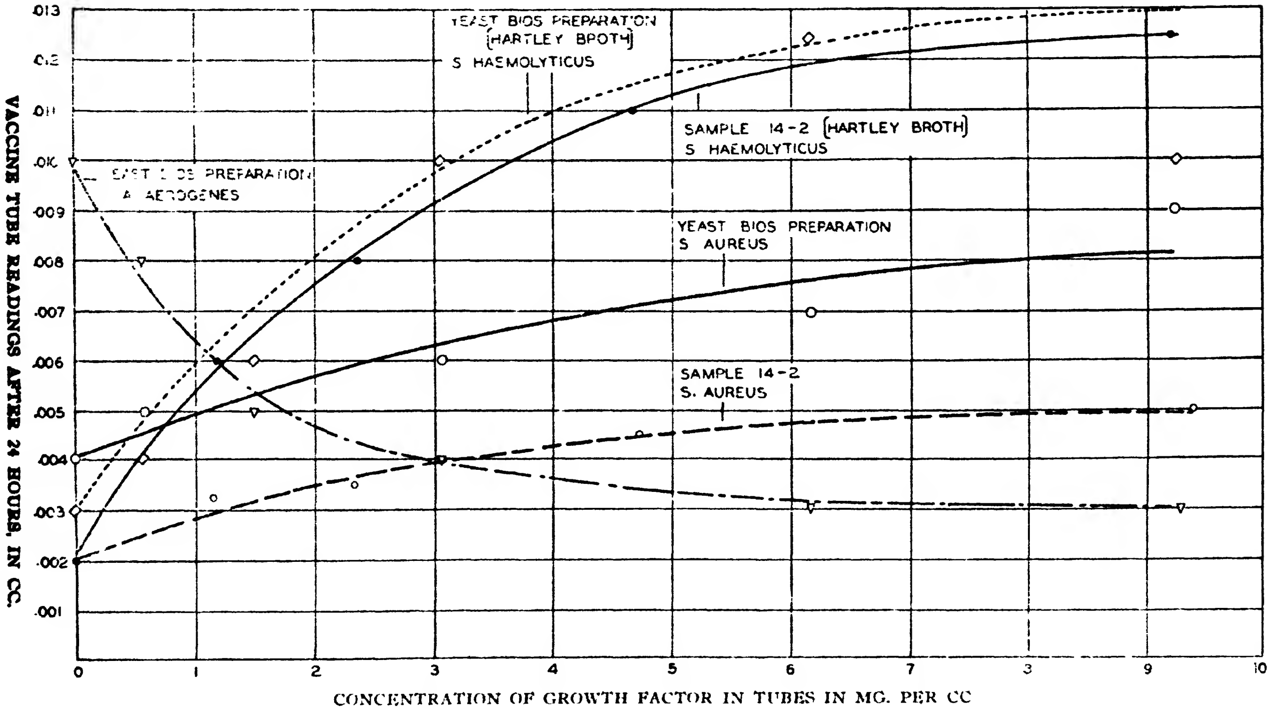

TABLE II

Summary of Experiments on Effects of Growth Factors on Bacteria

Organism	Growth Factors					
	Yeast "Bios" Preparation 12.4 mg./cc. 5.2 growth units/mg.	*Sample 8* Malt Combings Preparation 9.5 mg./cc. 1.09 respiration units/mg. 0.38 growth units/mg.	*Sample 14-1* Malt Combings Preparation 9.5 mg./cc. 1.5 respiration units/mg. 10.6 growth units/mg.	*Sample 14-2* Malt Combings Preparation 9.5 mg./cc. 1.5 respiration units/mg. 6.2 growth units/mg.	Irradiated Yeast Preparation 8.6 mg./cc. 1.05 growth units/mg.	Non-irradiated Yeast Preparation 8.6 mg./cc. 0.1 growth units/mg.
Staphylococcus aureus	3.5x	1.5x	3.0x	2.5x	5.0x	1.0x
Streptococcus haemolyticus	Hartley Broth 4.0x Nutrient Broth 1.0x	Hartley Broth 1.5x	Nutrient Broth 1 0x	Hartley Broth 7.0x		
Aerobacter aerogenes	0.7x	0.3x	0.9x	0 6x	3.0x	1.0x
Alkaligenes faecalis	1.5x	0.5x	1.5x	1 5x		
Bacillus pyocyaneus	1.5x		1.0x	1.0x (12.9 mg./cc.)		
Diplococcus pneumoniae, Type III	Hartley Broth 4.0x		Nutrient Broth 1.0x	Hartley Broth 5 0x	Hartley Broth 2.8x	Hartley Broth 1.1x

The table gives the average values of the ratio of growth of the experimental cultures to that of the controls after 24 hours. The growth and respiration units shown at the heads of the columns are for yeast. The concentrations shown are those at which the factors were employed in the bacterial suspensions.

All of our conclusions have been based upon the later, more extended and more quantitative investigations, described below.

Relation of Concentration to Growth. Figure 1 illustrates the relation of concentration to growth as it was observed in typical experiments in the later series. The curves were plotted from averages of Hopkins' tube readings. The experimental points are as consistent as might be expected under the conditions of the experiments. In the case of the factors which stimulated growth, increase in concentration resulted, in general, in a less than proportional increase in growth (i.e., the curves are concave downward). Because of limitations in the quantities of materials available, we were not able to investigate whether further increases in concentration would have resulted ultimately in reduced stimulation or perhaps in depression, or whether reduction in concentration would have led to stimulating action in these cases in which depression only was obtained.

Summary of Later Experiments. The results of all experiments in the later series are summarized in Table II in terms of the average ratio of growth in the test series to that in the controls for the highest concentration of growth factor used in each case. Thus 4.0x in the tables represents growth four times as great as that in the controls, and 0.5x represents growth half that in the controls. The concentrations of growth factors at the heads of the columns are the final concentrations in the suspensions to which they were added, after inoculation with bacteria, addition of salt solution, etc.

As in the preliminary experiments, both stimulation and inhibition of growth were obtained. The four "bios" preparations were roughly parallel in their effects, as in the previously published results.[1] The present, more quantitative data indicate certain significant differences, however. Thus: (1) *Sample 8* inhibited the growth of *Alkaligenes faecalis* while the others stimulated it; (2) while *Sample 14-1* was twice as potent as the yeast "bios" preparation in the yeast assays, it showed less stimulation for *Staphylococcus aureus*; and (3) in the case of *Streptococcus haemolyticus* and *Diplococcus pneumoniae*, some of the preparations stimulated growth when the organisms were cultured in Hartley broth but not when they were cultured in nutrient broth.

Essential Difference of Yeast Wound Hormones. The preparation from non-irradiated yeast had no effect on the organisms on which it was tried, while that from irradiated yeast markedly stimulated them. It is particularly interesting that concentrates from irradiated yeast suspensions stimulated *Aerobacter aerogenes*, whereas this organism was uniformly inhibited by all of the "bios" preparations. Thus the yeast wound hormone seems either to contain a growth factor different from those in "bios" preparations, or to be free from some toxic factor present in the "bios" preparations which inhibited the growth of *Aerobacter aerogenes*, or both.

In general, the data indicate a multiplicity of growth factors for bacteria in the concentrates studied. A similar multiplicity of respiratory factors has been found in preparations of this type by comparing their respiratory stimulation on yeast, liver and animal epithelium.[8, 24]

Slight Growth Stimulation of a Highly Active Respiratory Factor. Sample 8, which is fairly high in respiratory stimulating effect, markedly inhibited the growth of two of the organisms and but slightly stimulated the growth of the other two on which it was tried. This suggests that it may be possible to isolate respiratory stimulating factors which have little or no effect in stimulating bacterial growth, for use in combination with antiseptics, as suggested in the introduction. Fractions which have high respiratory stimulating effect on animal

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[24] Ruddy, Sr. M. Veronita, O.P., *Arch. exptl. Zellforsch.*, in publication.

tissue should be tried on a wide range of pathogenic micro-organisms, the concentrations in which they will overcome the tissue-respiration depressing effect of various antiseptics and bactericides should be determined, and the antiseptics combined with respiration-stimulating factors in these concentrations should be compared with the antiseptics alone as to their bactericidal power and their inhibition of tissue growth *in vitro*. We believe that it would be wiser to undertake such an investigation after further work has been completed on the fractionation of these materials. Considerable progress has already been made along this line by Dr. E. S. Cook *et al.* of these laboratories, and this work is rapidly being extended.

CONCLUSIONS

The results described lead us to the following conclusions:

1. Various "bios" fractions prepared from yeast by the method of Narayanan and from malt combings by the method of Lucas stimulate the growth of certain micro-organisms and depress that of others within the concentration range of approximately 0.5 to 10 mg. per cc.

2. Differences in the effects of the different fractions on various micro-organisms indicate that there are several active substances present in the different preparations rather than a single substance common to all preparations.

3. The fact that the yeast wound hormone stimulates the growth of *Aerobacter aerogenes* while all of the "bios" fractions depress it indicates that the wound hormone contains a growth factor different from those in the "bios" preparations.

4. The low growth activity of a preparation high in respiratory stimulating activity suggests the possibility of obtaining fractions which will compensate the respiratory depressing effect of antiseptics without decreasing their bactericidal action.

LITERATURE CITED

Beck, W. A., *Science*, 85 (1937): 368.

Cook, E. S., Kreke, C. W., and Nutini, L. G., This Issue, p. 23.

Cook, E. S., and Kreke, C. W., This Issue, p. 47.

Elder, M. L., *Trans. Roy. Soc. Can.*, 33 (1936): 89.

Fardon, J. C., Carroll, Sr. M. Jordan, O.P., Ruddy, Sr. M. Veronita, O.P., These Studies, 1 (1937): 17.

Fardon, J. C., Norris, R. J., Loofbourow, J. R., and Ruddy, Sr. M. Veronita, O.P., *Nature*, 139 (1937): 589.

Fardon, J. C., and Ruddy, Sr. M. Veronita, O.P., These Studies, 1 (1937): 41.

Holiday, E. R., *Biochem. J.*, 31 (1937): 1299.

Knight, B. C. J. G., *Biochem. J.*, 31 (1937): 731; *Nature*, 139 (1937): 628; *J. Soc. Chem. Ind.*, 56 (1937): 445.

Koser, S. A., and Saunders, F., *J. Bact.*, 29 (1935): 17.

Koser, S. A., Saunders, F., Finkle, I. J., and Spoelstra, R. C., *J. Infectious Diseases*, 58 (1936): 121, 127.

Loofbourow, J. R., Cueto, A. A., and Lane, Sr. M. Michaella, S.C., *Arch. exptl. Zellforsch*, in publication.

Loofbourow, J. R., Dwyer, Sr. Cecelia Marie, S.C., and Morgan, Sr. M. Norbert, R.S.M., This Issue, p. 137.

Loofbourow, J. R., Norris, R. J., and Morgan, Sr. M. Norbert, R.S.M., These Studies, 1 (1937): 193.

Lwoff, A., and Lwoff, M., *Proc. Roy. Soc.* (*London*), B-122 (1937): 352.

Lucas, G. H. W., *J. Phys. Chem.*, 28 (1924): 1180.

Narayanan, B. T., *Biochem. J.*, 24 (1930): 6.

Norris, R. J., and Hart, Sr. M. Jane, O.P., These Studies, 1 (1937): 65.

Norris, R. J., and Kreke, C. W., *ibid.*, 1 (1937): 137.

Norris, R. J., and Ruddy, Sr. M. Veronita, O.P., *ibid.*, 1 (1937): 53.

Pasteur, L., Studies on Fermentation, Paris (1876): 331.

Pulkki, L. H., *Ann. Acad. Sci. Fennicae*, A-41 No. 1 (1935): 141.

Reader, V., *Biochem. J.*, 21 (1927): 901.

Richardson, G. H., *Biochem. J.*, 30 (1936): 2184.

Ruddy, Sr. M. Veronita, O.P., *Arch. exptl. Zellforsch.*, in publication.

Schopfer, W. H., *Arch. Mikrobiol.*, 5 (1934): 502, 511; 6 (1935): 139, 196, 334; *Compt. rend.*, 200 (1935): 1965; *Ber. deut. botan. Ges.*, 54 (1936): 559; *Z. Vitaminforsch.*, 4 (1937): 187.

Schopfer, W. H., and Jung, A., *Arch. Mikrobiol.*, **6** (1935): 345; *Compt. rend.*, **204** (1937): 1500.

Sinclair, H. M., *Nature*, **140** (1937): 361.

Snell, E. E., Tatum, E. L., and Peterson, W. H., *J. Bact.*, **33** (1937): 207.

Sperti, G. S., Loofbourow, J. R., and Dwyer, Sr. Cecelia Marie, S.C., *Nature*, **140** (1937): 643; THESE STUDIES, **1** (1937): 163.

Sperti, G. S., Loofbourow, J. R., and Lane, Sr. M. Michaella, S.C., *Science*, **86** (1937): 611.

Tanner, F. W., *Chem. Rev.*, **1** (1925): 397.

Tatum, E. L., Wood, H. G., and Peterson, W. H., *Biochem. J.*, **30** (1936): 1898; *J. Bact.*, **32** (1936): 167.

Tatum, E. L., Peterson, W. H., and Fred, E. B., *J. Bact.*, **32** (1936): 157.

Wildiers, E., *La Cellule*, **18** (1901): 313.

Wood, H. G., Tatum, E. L., and Peterson, W. H., *J. Bact.*, **33** (1937): 227.

Original Manuscript submitted
July 10, 1938.

RELATIVE CONSISTENCY OF WEIGHTS AND COUNTS IN DETERMINING MICROORGANISM POPULATIONS BY PHOTOELECTRIC NEPHELOMETERS

By JOHN R. LOOFBOUROW and
SISTER CECELIA MARIE DWYER, S.C.

THE advantages of photoelectric nephelometers for determining the population density of microorganisms have been emphasized by several workers, and a number of devices suitable for such measurements have been described in the literature.[1] Some investigators have assumed that, if conditions of ages of cultures, etc., are sufficiently well standardized, the number of microorganisms present in unit volume, C, can be taken as proportional to the optical density, D, of a suspension of thickness *l*, thus:

$$C = AD \text{ where } D = \log_{10} \frac{I_0}{I_x}$$

The latter expression holds true theoretically only for the absorption of monochromatic radiation by homogeneous solutions of absorbing materials, and the former holds only if all cells are uniform in their absorbing properties. None of these conditions are fulfilled in practice: the density determinations are made on suspensions instead of solutions (in which scattering of light as well as absorption plays an important part); monochromatic radiation is seldom employed; and variations in the size and optical properties of the microorganisms under various conditions cause the absorption per cell to vary within wide ranges. Such perturbing factors are of interest, however, only in so far as they lead to inconsistencies in the results too great to be ignored.

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[1] Peskett, G. L., *Biochem. J.*, 21 (1927): 460; Richards, O. W., and Jahn, T. C., *J. Bact.*, 26 (1933): 385; Mestre, H., *J. Bact.*, 30 (1935): 335; Stier, T. J. B., Arnold, W., and Stannard, J. N., *J. Gen. Physiol.*, 17 (1934): 383; Beck, W. A., *Science*, 85 (1937): 368.

The authors were interested in the measurement of the population densities of cultures of yeast and other microorganisms in testing the growth-promoting effects of various substances. For this purpose, some convenient method was required which would give results reproducible to within a few per cent. Employing Beck's photodensitometer,[2] with a Wratten 49C blue glass filter, a comparison was made between the optical densities of various yeast suspensions, the number of microorganisms present as determined by haemacytometer counts, and the wet weight of the yeast in suspension. Readings by various observers were compared. It was found that a much closer correlation existed between wet weight and optical density under the conditions of these experiments than between cell count and optical density.

Thus Fig. 1 shows a comparison of the counts with percentage transmissions for four series of dilutions made from Fleischmann's bakers' yeast.* The haemacytometer counts for each dilution were averaged, multiplied by the dilution, and the average of these values taken as the count of the undiluted suspension. Counts of the other suspensions were calculated from this value and the dilution. The actual haemacytometer counts for several dilutions are shown on Curve 1. The variation in the counts corresponding to the same densities was as great as 4 to 1. Figure 2 shows a similar series of dilution curves obtained for yeast grown in liquid Sabouraud's medium from a strain of *Saccharomyces cerevisiae* obtained from Fleischmann's bakers' yeast. The yeast culture was centrifuged, filtered, and the yeast residue washed and suspended in isotonic

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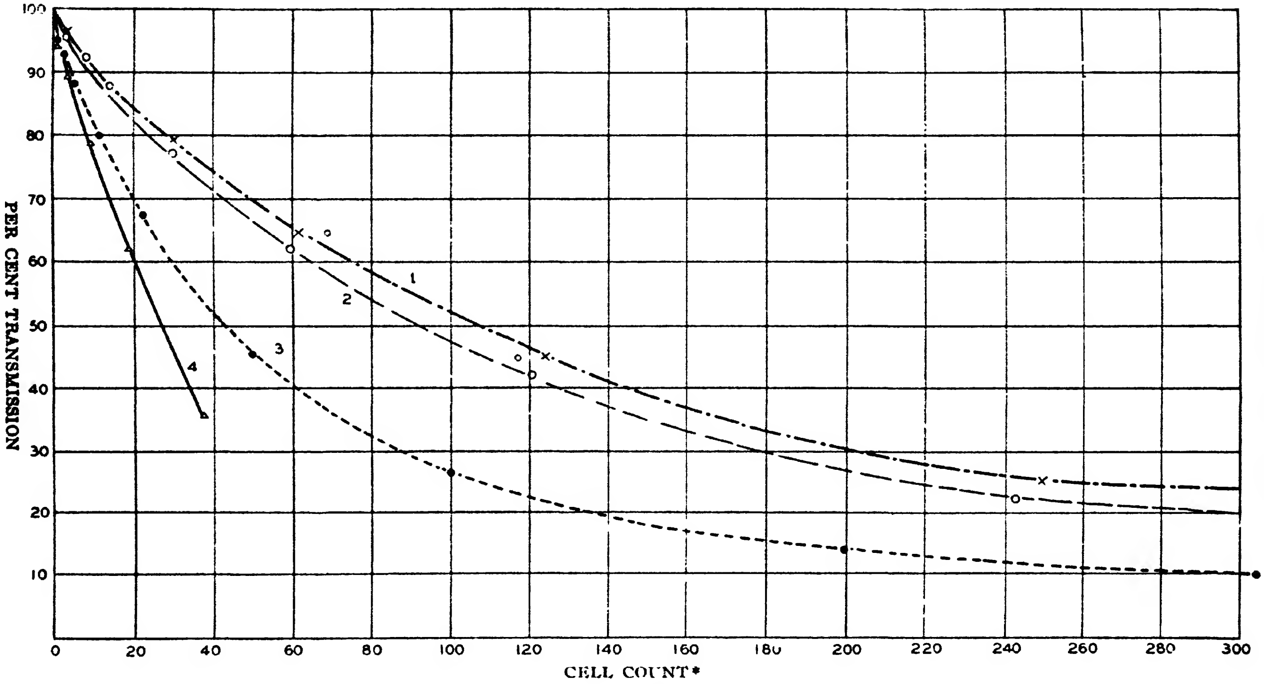
[2] Beck, W. A., *loc. cit.*

*Each series of dilutions was made from a different sample of yeast.

FIG. 1—*Dilution Curves of Fleischmann's Bakers' Yeast on Basis of Cell Count.*



The points on the curves were calculated from the averages of haemacytometer counts for several dilutions, each multiplied by the dilution. The circles neighboring Curve 1 are actual values of individual haemacytometer counts.

*Count × 250 = Cells per cu.mm.

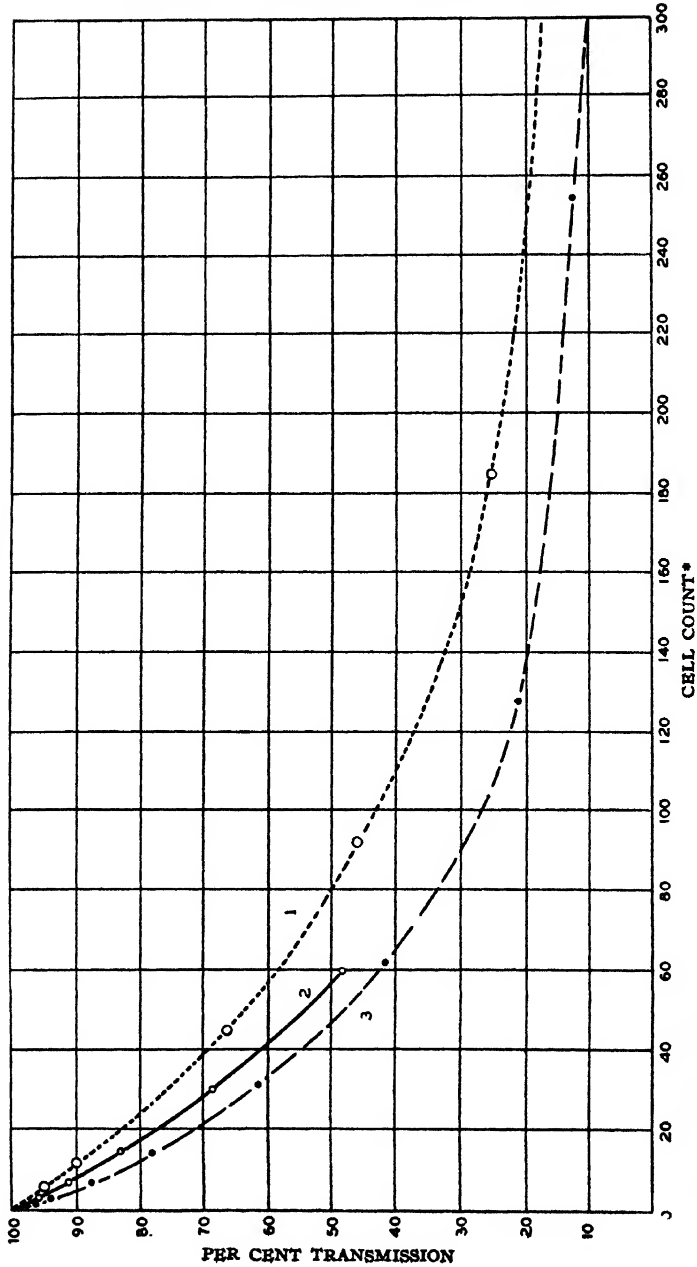


Fig. 2—*Dilution Curves of Saccharomyces Cerevisiae, Grown in Liquid Sabouraud's Medium, on Basis of Cell Count.*

*Count × 250 = Cells per cu.mm.

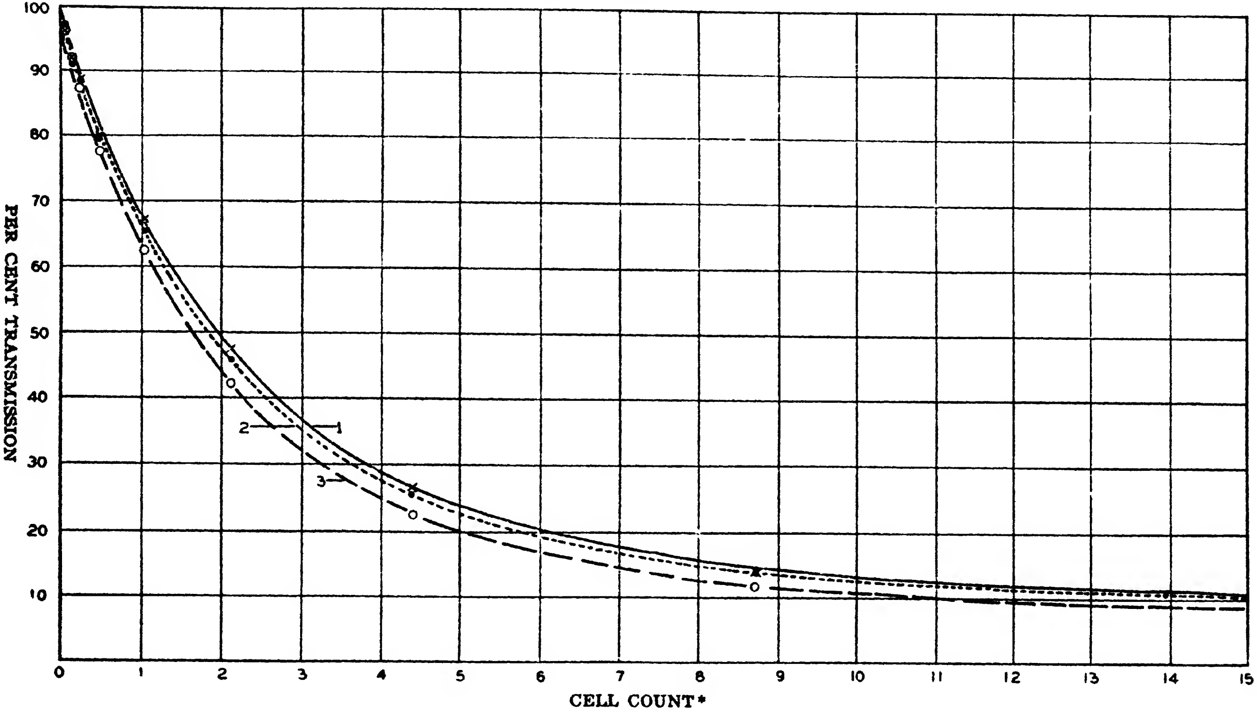
salt solution. Throughout the experiments conditions were kept as uniform as practicable. While the variation in these suspensions was not as great as in suspensions made from cake yeast (due probably to greater uniformity of size and age of cells), the inconsistency between counts and transmission values was still quite marked. In both instances repeated observations by various observers were consistent to within a small fraction of the differences between the curves.

Since the absorption should be more closely correlated with the total amount of protoplasm present than with cell number, because of variation in the size of cells, a comparison of the wet weight of yeast suspended in solution with the density readings was made. Figure 3 shows a series of curves obtained by diluting known weights of compressed yeast in salt solution. Curves 1, 2 and 3 correspond to the similarly numbered curves in Fig. 1, the same suspensions having been used for both series of data. The consistency of the curves in Fig. 3, while still leaving much to be desired, is so much better than that obtained when counts and densities are compared that the advisability of using weight instead of count in expressing population density values obtained by photodensitometer readings is apparent.

It is possible that greater precautions in obtaining uniform growth conditions might lead to better consistency between counts and densities, but what is desired is a method of expressing population densities, as determined by photoelectric methods, which will yield reasonably consistent values without the necessity of such extraordinary precautions.

Even on a wet weight basis, the maximum variation of weight with transmission is of the order of 10 per cent for the data shown. These are extreme values for cake yeast of different ages, and greater consistency can be obtained in cultured yeast grown under carefully controlled conditions. Nevertheless it is probably not safe when comparing values

FIG. 3—*Dilution Curves of Fleischmann's Bakers' Yeast on Basis of Weight*



Compare Curves 1, 2, 3, of Figure 1.

*Count × 250 = Cells per cu.mm.

from different experiments to consider as significant, variations of less than 10 per cent on a wet weight basis or 50 per cent on a cell-count basis.

Experiences in these laboratories with calibrations on a dry weight basis have so far not been any more satisfactory than with those on a wet weight basis, but it may be that improvements in the technique of drying will lead to more consistent results.

It is unfortunate for investigations of growth factors that the photoelectric method, as is evident from the data, does not give a criterion for cell number as distinct from cell size. However, parallel checks with haemacytometer counts enable one to determine in any individual series of experiments whether increase in photodensitometer reading is a result of increase in cell size alone or of increase in cell number as well.

Because of the high absorption of microorganisms in the ultraviolet region, especially beyond 3000 Å, the use of ultraviolet light should make possible the estimation of populations in much less dense suspensions than one must use when employing visible radiation. The possible advantages of photodensitometers employing ultraviolet sources are being investigated.*

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*Thanks are due Dr. Elton Cook, Dr. Andre Cueto, Dr. Robert Norris, Mr. Cornelius Kreke, and Sister Mary Norbert Morgan, R.S.M., for their assistance in comparing the readings obtained by various observers.

Original Manuscript submitted
June 15, 1938.

INTERCELLULAR WOUND HORMONES FROM ULTRAVIOLET INJURED CELLS

By JOHN R. LOOFBOUROW, SISTER CECELIA MARIE DWYER, S.C., AND SISTER MARY NORBERT MORGAN, R.S.M.

The wound hormone hypothesis postulated by Wiesner[1] to account for the proliferation of plant cells in the neighborhood of injured tissues has been the object of considerable inquiry. In particular, Haberlandt[2] and Reiche[3] were able to show that crushed plant tissues placed on plant wounds favored the repair process.

In the case of animal wounds, Carrel emphasized in his earlier papers the importance of leucocytic secretions, which he called "trephones".[4] More recently[5] he has cited evidence to show that substances set free from the traumatized cells themselves play an important part in the initial stimulus to wound healing.

Growth-stimulating extracts have been prepared from cells and tissues by various workers, and some have sought to identify these principles with wound hormones. Thus Bonner[6] and Bonner and English[7] recently extracted a growth factor from plant tissues which they believe to be a wound hormone, and Hammet[8] has concluded from studies of proliferation-promoting factors that the sulfhydryl radical is the active principle in the stimulation of wound repair. The identification of such materials with wound hormones rests, however, on the analogy of their proliferation-promoting effects. Bonner and English found that injury to the plant tissues did not increase the yield of the material they were extracting.

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[1] Wiesner, J., Elementarstructur, Vienna (1892), p. 102.

[2] Haberlandt, G., *Beitr. allgem. Botan.*, 2 (1921): 1; *Sitz. preuss. Akad. Wiss.* (1921): 221; *Biol. Zentr.*, 42 (1922): 145.

[3] Reiche, H., *Z. Botan.*, 16 (1924): 241.

[4] Carrel, A., *J. Am. Med. Assoc.*, 82 (1924): 255, and previous publications referred to in his paper.

[5] Carrel, A., *Proc. Inst. Med., Chicago*, 8 (1930): 62.

[6] Bonner, J., *Science*, 85 (1937): 183.

[7] Bonner, J., and English, J., *J. Biol. Chem.*, 121 (1937): 791.

[8] Hammet, F., *Protoplasma*, 13 (1931): 331.

Recently, new techniques employed in our laboratories[9] have made it possible to obtain growth-promoting extracts from plant and animal cells in which the yield has been shown to be correlated with the injury to which the cells were subjected before or during extraction. We do not know of any evidence previously reported in the literature in which a definite relationship between cellular injury and the production of proliferation-promoting extracts has been demonstrated. In the investigations which we have reported, yeast[10] and animal tissues[11] were injured by prolonged irradiation with full ultraviolet, or by other means. The intercellular fluids were freed from cells (usually by filtration through bacterial filters) and the cell-free fluids were compared as to their proliferation-promoting potencies with fluids from controls which had stood the same length of time without being subjected to injury.

In the yeast experiments, small agar blocks were inoculated on the top with yeast. Cover slips were placed over them and sealed to them with additional agar. Injured or uninjured cells, or intercellular fluids from suspensions of such cells, were added to the bottom of the blocks, and the cover slips and blocks were placed on hollow-ground slides. Proliferation-promoting factors diffused from the bottom of the blocks through the agar and influenced the growth of the inocula on the top. The areas of growth were measured after 24 and 48 hours. Addition of injured cell suspensions, or intercellular fluids therefrom, always resulted in marked increase in growth as compared with the addition of uninjured cells or corresponding intercellular fluids.

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[9] Fardon, J. C., Norris, R. J., Loofbourow, J. R., and Ruddy, Sr. M. Veronita, O.P., *Nature*, 139 (1937): 589.

[10] Sperti, G. S., Loofbourow, J. R., and Dwyer, Sr. Cecelia Marie, S.C., *Nature*, 140 (1937): 643; THESE STUDIES, 1 (1937): 163.

[11] Sperti, G. S., Loofbourow, J. R., and Lane, Sr. M. Michaella, S.C., *Science*, 86 (1937): 611; Loofbourow, J. R., Cueto, A. A., and Lane, Sr. M. Michaella, S.C., *Z. Zellforsch. mikroskop. Anat.* In publication.

Such experiments, while simple and effective, are not sufficiently quantitative to afford data as to the relative potencies and yields obtained in various preparations. We wish to report here the results of investigations, by a more quantitative method, which have enabled us to learn something further regarding the properties of the wound hormones and the conditions relating to their production.

EXPERIMENTAL METHODS

The wound hormones were produced by the irradiation of yeast (*Saccharomyces cerevisiae*) with full ultraviolet light. This affords a convenient and easily controllable method of injury. Staining with methylene blue was used as a criterion of cellular injury.[12]

Compressed yeast was suspended in isotonic salt solution in concentrations varying from 17.5 to 100 mg. per cc. The suspensions were divided into two portions, one of which was irradiated with full ultraviolet at 25 cm. from a Burdick quartz mercury arc operated at 65 volts. The other was kept as a control suspension at a comparable temperature throughout the period of irradiation. After irradiation, both suspensions were centrifuged or filtered through Büchner filters, to remove the yeast, after which they were filtered through Berkefeld filters to free them from any remaining cells. The yields were calculated by taking the filtrates to dryness, weigh-

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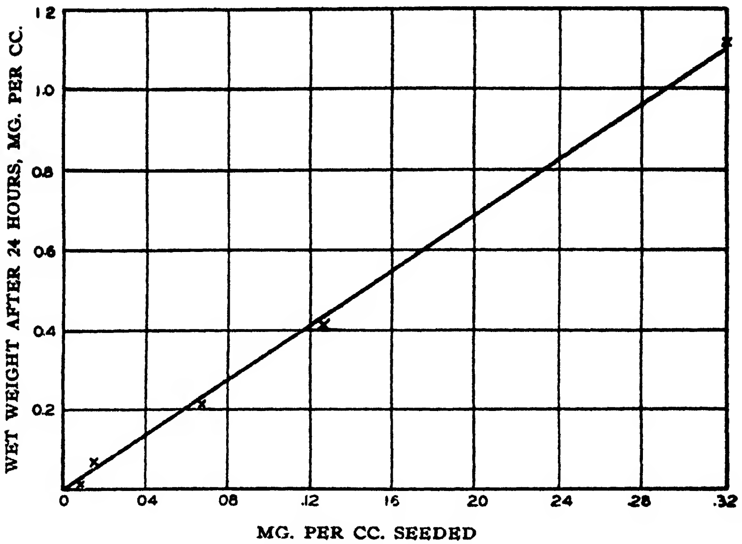
[12] Richards, O. W., *Archiv. Protistenkunde*, 78 (1932): 263.

FIG. 1—*Effect of Seeding on 24-Hour Crop of Yeast in Rocker Tubes*



ing the material so obtained, and subtracting the weight of the salt present.

The materials were assayed by adding them to cultures of yeast. These were grown in Reader's medium[13] in rocker tubes[14] for 24 hours at 30° C. Each tube contained a total of 25 cc. (including added material) and was seeded with 0.064 mg. per cc. of cultured yeast. This quantity was used because it corresponded to the amount employed by Norris, Kreke,[15] Cook *et al.*[16] in their assays of various "bios" preparations.

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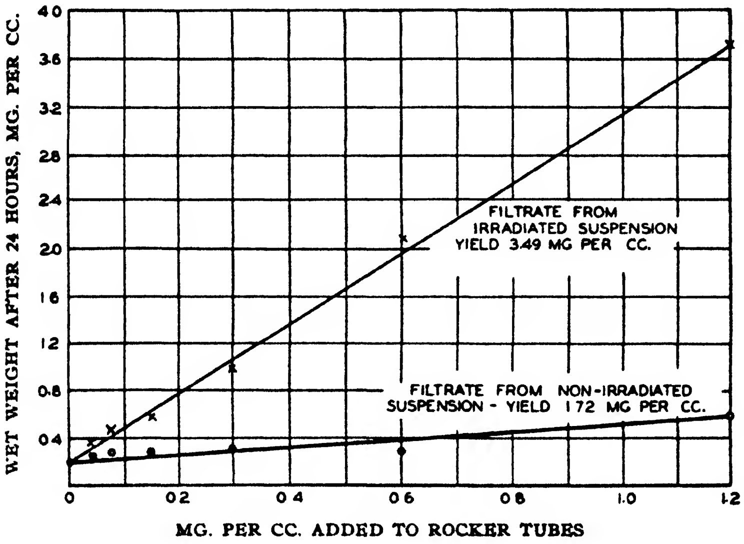
[13] Reader, V., *Biochem. J.*, 21 (1927): 901.

[14] Fraser, C. G., *J. Phys. Chem.*, 25 (1921): 4; Lucas, G. H. W., *J. Phys. Chem.*, 28 (1924): 1180; Norris, R. J., and Hart, Sr. M. Jane, O.P., THESE STUDIES, 1 (1937): 65.

[15] Norris, R. J., and Kreke, C. W., THESE STUDIES, 1 (1937): 137.

[16] Cook, E. S., Kreke, C. W., and Nutini, L. G., THIS ISSUE, p. 23.

FIG. 2—*Assay of 1/7/38 Preparation After Taking to Dryness*



In order to see what variations might be introduced by inaccuracies in seeding, we investigated the question of the relation of seeding to the yeast crop after 24 hours. The results, plotted in Fig. 1, show that within the range studied the crop is directly proportional to the seeding.

The yeast crops after 24 hours were determined by a photoelectric photodensitometer.[17] Since this measured total protoplasm present rather than number of cells,[18] all of our data are given in terms of yield on a weight basis (wet weight of yeast in mg. per cc.), rather than in population counts. Microscopic examination of the rocker-tube contents after the growth period showed, however, that the average size of cells was no greater than that in the original inocula (frequently it was less because of the presence of many young cells);

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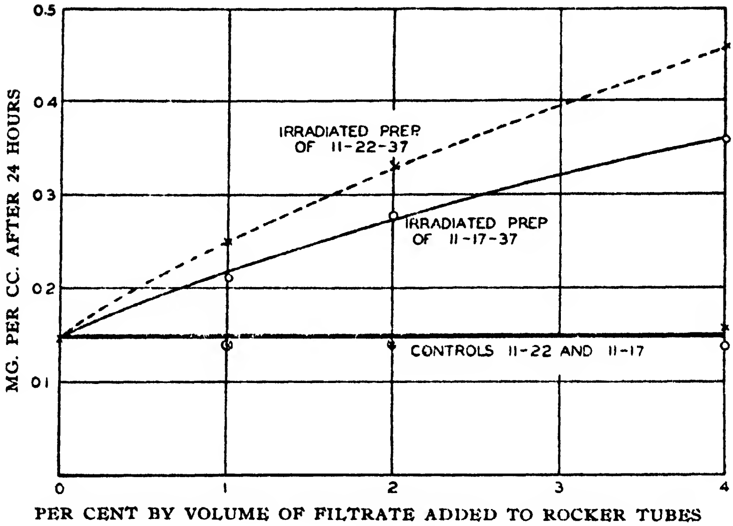
[17] Beck, W. A., *Science*, 85 (1937): 368.

[18] Loofbourow, J. R., and Dwyer, Sr. Cecelia Marie, S.C., THIS ISSUE, p. 129.

FIG. 3—*Typical Results of Assays of Filtrates Before Concentration*



hence increased crop weight represented stimulation of proliferation rather than of growth in cell size.

As a method of expressing the proliferation-promoting potencies of the samples assayed, we adopted the "growth unit" used by Norris and Kreke[15] in their "bios" assays. This they defined as an increased yeast crop after 24 hours, corresponding to a count of 25 greater than that in the control tubes.* On our weight basis, this corresponded to a concentration of 1.6 mg. per cc. greater than that in the controls. The number of growth units per mg. of material added to each cc. of rocker tube contents was calculated from curves relating the crop after 24 hours to the concentration of added material. Figure 2 is typical of such curves. It is to be noted that within the range of concentrations we employed, the relationship between crop and amount of material added was practically linear. This facilitated the calculation of potencies on a growth unit basis. Norris and Kreke obtained distinctly different types of curves in their assays of "bios" preparations.[15]

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*There is some question as to whether or not this is the best way of defining growth units, but we have used this unit in the present calculations in order to facilitate comparison with earlier work.

EXPERIMENTAL RESULTS

Assays of Unconcentrated Filtrates. In the early experiments, the filtrates from control and irradiated suspensions were added directly to the rocker tubes in amounts of 1 cc. (4 per cent by volume) or dilutions thereof. In such concentrations, filtrates from the non-irradiated suspensions had no measurable effect on the yeast crop, while those from the irradiated suspensions stimulated growth by as much as 200 per cent (Fig. 3). In these experiments, the yeast was irradiated until approximately 90 per cent of the cells were stained. This required about one hour at the concentration and thickness of suspensions used (17.5 mg. per cc., 2.5 cm. stratum).

These results clearly show the production of proliferation-promoting factors (wound hormones) in the irradiated suspensions. They do not permit quantitative comparison, however, with the filtrates from non-irradiated suspensions. One might have concluded from these experiments that the control filtrates were not potent, but when these were taken to dryness and added in higher concentration, they showed some proliferation-promoting effect, as illustrated in the data which follow.

Effect of Drying and Autoclaving. The most convenient method of studying the effects of the materials in higher concentrations was to take them to dryness and then make the desired concentrations. This necessitated an investigation of the effect of drying on the potencies of the active materials. Repeated assays of the filtrates before they were taken to

TABLE I

Typical Results of Autoclaving Active Filtrates for 15 Minutes at 20 Pounds

Preparation	Time of Irradiation	Growth Units per cc. of Filtrate	
		Before Autoclaving	After Autoclaving
2/17/38 A	4.5 hr. (25 per cent stained)	3 38	3.34
2/17/38 A	Non-irradiated Control	0.79	0.77

dryness and after they were dried on a water bath, or in an oven at about 65° C., showed no appreciable loss in activity. Thereafter the majority of samples were taken to dryness before they were assayed, though samples of the original filtrates were tested periodically as a precautionary procedure.

The effect of autoclaving the filtrates at 20 pounds for 15 minutes was tried repeatedly. Typical results are shown in Table I. No significant reduction in activity resulted from autoclaving.

Comparison of Büchner Filtration and Centrifugation. The potencies of the control filtrates seemed to be greater if the suspensions were filtered through a Büchner filter before Berkefeld filtration instead of being centrifuged first (Table II). The explanation of this is not known, unless it is that the cells were subjected to greater or more prolonged injury in the Büchner filtrations. Similar differences were not noted in the filtrates from the irradiated suspensions.

Are the Factors Degeneration Products or Secretions of Living Injured Cells? The question as to whether the proliferation-promoting factors from the irradiated suspensions are hormone-like substances released by injured cells into the intercellular fluid or simply cellular destruction products has seemed to us to be of considerable importance. It is a difficult problem to investigate because cellular destruction products, such as proteins and their amino acid derivatives, would be expected

TABLE II

Comparison of Potencies of Control Filtrates in which Cells were Removed by Centrifuging and by Büchner Filtration

Preparation	Method of Separation	Concentration of Filtrates mg./cc.	Growth Units per mg.	Growth Units per cc. of Filtrate	Growth Units per g. of Yeast
Non-irradiated Control Suspension 2/17/38 A and B 100 mg. per cc.	Centrifuge	2 39	0.095	0 23	2 3
	Büchner Filter	2 32	0.34	0.79	7.9

to have a growth-promoting effect. Our investigations lead us to believe, however, that such products play, if anything, only a minor role in the effects obtained, and that the proliferation-promoting factors produced as a primary result of injury are actually hormone-like substances elaborated by living cells as a physiologic response to injury and released into the intercellular fluid.

These conclusions are based upon the following observations: (1) in even the most prolonged periods of irradiation employed in the experiments reported here, microscopic examination of smears failed to reveal any evidence of cytolysis; (2) the active, cell-free filtrates were free from protein (biuret test);[19] (3) cells killed by autoclaving before irradiation did not show measurable production of the active factors when irradiated; (4) appreciable increase in potency of filtrates from irradiated suspensions as compared with control filtrates was obtained before there was any evidence of killing (increased staining) in the irradiated suspensions; (5) when the cells were suspended in an unfavorable medium, such as distilled

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[19] Cook, E. S., Loofbourow, J. R., and Stimson, Sr. M. Michael, O.P., Tenth International Congress of Chemistry, Rome, Italy, May, 1938, to be published in the Proceedings of the Congress; Loofbourow, J. R., Cook, E. S., and Stimson, Sr. M. Michael, O.P., *Nature*, **142** (1938): 573.

TABLE III

Results Illustrative of Higher Yields Obtained by Prolonged, Mild Injury

Preparation	Concentration of Suspensions in mg./cc.	Irradiation Time in hr.	Concentration of Filtrate in mg./cc.	Growth Units per mg.	Growth Units per cc. of Filtrate	Growth Units per g. of Yeast
A	50	1.5	3.49	1.84	6.42	128
B	100	7	14.3	5.0	71.5	715

Filtrates from Non-irradiated Control Suspensions Corresponding to Above

A	50	Non-irradiated Less than 1% stained	1.72	0.21	0.36	7.2
B	100	Non-irradiated 1 to 2% stained	0.59	1.39	0.82	8.2

In each case the control suspensions stood during the time of irradiation under similar conditions of temperature, etc. The differences in time of irradiation required for 90-100 per cent staining were obtained by varying the depth and concentration of the suspensions irradiated. All suspensions were filtered through Berkefeld filters.

TABLE IV

Comparison of Various Irradiated Yeast Preparations and "Bios" Preparations

Preparation	Concentration of Suspension in mg./cc.	Time Required for 90-100% staining in min.	Concentration of Filtrates in mg./cc.	Growth Units per mg.	Growth Units per cc. of Filtrate	Growth Units per g. of Yeast
11/17/37	17.5	60	1.59	2.06	3.28	188
		Non-irradiated Control	0.77	*	*	*
11/22/37	17.5	55	1.78	2.72	4.67	267
		Non-irradiated Control	0.26	*	*	*
12/18/37	50	90	3.75	2.08	7.7	154
		Non-irradiated Control	1.13	*	*	*
12/20/37	50	90	3.21	2.03	6.52	134
		Non-irradiated Control	1.85	*	*	*
1/7/38	50	90	3.49	1.84	6.42	128
		Non-irradiated Control	1.72	0.21	0.36	7.2
4/26/38	100	420	14.30	5.0	71.5	715
		Non-irradiated Control	0.59	1.39	0.82	8.2
Crude Narayanan "Bios" Preparation from Yeast.			Yield 3.63 mg./ 100 mg. yeast	3.43		125
Narayanan Yeast "Bios" Preparation 80% Alcohol Extraction of Lead Acetate Filtrate.			Yield 0.404 mg./ 100 mg. yeast	5.2		21

*Not measurable.

water, the potencies of the control filtrates were higher but those of the filtrates from irradiated cells were lower than when the cells were suspended in isotonic solutions; (6) when the injury was prolonged at low intensities, the yield was higher than when the same degree of injury was applied over a shorter time. The evidence concerning the first five of these observations will be discussed in subsequent papers. We wish to give attenion here only to the sixth.

Table III illustrates the higher yields obtained by prolonged, low-intensity injury. The results tabulated are typical of those obtained in a large number of experiments. The ultraviolet source and the irradiation distance were the same in both instances. The concentration and depth of the yeast suspensions were varied, however, so that in preparation "A", more than 90 per cent of the cells were stained in 1½ hours, whereas in preparation "B", 7 hours of irradiation were required to produce the same degree of staining. The per cent of cells killed (stained) at the end of the irradiation period was the same in both instances. While in preparation "B" a suspension of only twice the concentration was used, the yield per cc. of filtrate was approximately four times as great and the potency per mg. of material three times as great. Thus the yield in terms of growth units per gram of yeast in suspension was roughly six times as great in the long irradiation at low rate of injury. That this cannot be accounted for by the longer period of extraction is indicated by a comparison of the yields, in growth units per mg. of yeast, in the control suspensions (Table III). The long period of standing resulted in an increase in yield of only about 15 per cent.

Range of Concentration Effective. A summary of the yields and potencies obtained in several preparations, together with similar data for two "bios" preparations prepared by Narayanan's method[20] from yeast, is given in Table IV.

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[20] Narayanan, B. T., *Biochem. J.*, **24** (1930): 6.

The yield obtained from non-irradiated control suspensions averaged about 8 growth units per gram of yeast in suspension. Those from irradiated suspensions ranged from 128 to 715 growth units per gram of yeast. Thus the potencies per gram of yeast following irradiation were 16 to 90 times as great as those obtained in controls, ratios sufficient to indicate a definite effect even with much cruder methods of assay.

The concentrations of the crude material from irradiated suspensions used in the rocker tube experiments ranged from 1:175 to 1:26,000 (by weight). Stimulation was obtained throughout this range.

The smallest concentration of preparation 4/26/38 (the most potent of those listed in Table IV) used in rocker tube experiments was 0.032 mg. per cc. Since this produced roughly four times the least increase in growth measurable by the method, it is evident that 8 γ of this material per cc. (a concentration of 1:125,000 by weight) should show noticeable activity with the method of assay used.

This preparation (4/26/38) was more potent on a weight basis than a crude "bios" preparation made by combining the 95 per cent and 50 per cent alcohol extractions of the first step of Narayanan's method (Table IV). It gave roughly five times the yield in growth units per gram of yeast as the "bios" preparation. It was practically as potent, weight for weight, as a more highly purified Narayanan "bios" preparation,[21] listed in the table, and gave somewhat greater yield in growth units per gram of yeast.

Solubility of Active Material. The solubility of the active material in various solvents was investigated by attempting to dissolve 300 mg. portions of a dried filtrate from an irradiated suspension, previously assayed at 30 mg. per cc., in 10 cc. each of various solvents, filtering to remove undissolved material, taking the filtrates to dryness, and dissolving each dried filtrate in 10 cc. of distilled water. Care was taken to suspend all dried material in the water, and to see that suspended as well as dissolved material was added to the rocker tubes. Assays of the final water suspensions indicated that the active material is soluble in water and 50 per cent alcohol, slightly soluble in 95 per cent and absolute alcohol, and insoluble in ether and petroleum ether.

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[21] Loofbourow, J. R., and Morgan, Sr. M. Norbert, R.S.M., THIS ISSUE, p. 113.

Further Investigations. Preliminary studies of the active filtrates by absorption spectra and chemical tests[19, 22] have indicated that they are free from protein, halogens, or sulphur, and that they contain guanine, adenine, pentose, phosphorus, but not nicotinic acid or amide. Their apparent relation to nucleic acid derivatives is interesting in view of the work of Calkins[23] and Calkins, Bullock and Rohdenburg,[24] who showed many years ago that certain nucleic acid derivatives stimulated cellular proliferation, and obtained similar biologic effects with materials from autolyzed cells.

Data concerning the production of the wound hormones by other methods of injury will appear subsequently. Results recently published[25] indicate that biologically similar factors are produced by the toxic action of heteroauxin on yeast.

Preliminary evidence that the active filtrates stimulate respiration and fermentation as well as proliferation has been published.[26] An investigation of the relationship between

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[22] Loofbourow, J. R., Schmieder, L., Stimson, Sr. M. Michael, O.P., and Dwyer, Sr. Cecelia Marie, S.C., THESE STUDIES, 1 (1937): 79; Loofbourow, J. R., Cook, E. S., and Stimson, Sr. M. Michael, O.P., *Nature*, 142 (1938): 573.

[23] Calkins, G. N., *Proc. Soc. Exptl. Biol. Med.*, 9 (1912): 97.

[24] Calkins, G. N., Bullock, F. D., and Rohdenburg, G. L., *J. Infectious Diseases*, 10 (1912): 421.

[25] Loofbourow, J. R., and Dwyer, Sr. Cecelia Marie, S.C., *Science*, 88 (1938): 191.

[26] Fardon, J. C., and Ruddy, Sr. M. Veronita, O.P., THESE STUDIES, 1 (1937): 41; Norris, R. J., and Ruddy, Sr. M. Veronita, O.P., *ibid.*, 1 (1937): 53.

time of irradiation, killing of cells as determined by staining and plating, production of proliferation, respiration and fermentation-stimulating factors, and changes in ultraviolet absorption spectra is in progress. Recently, Harker[27] has found that the exposure of yeast suspensions to radium radiation results in the production of materials in the intercellular fluid stimulating sucrose inversion.

The growth, fermentation and respiration studies now in progress, in cooperation with our colleague, Dr. E. S. Cook, seem to indicate thus far, on the basis of comparison with various "bios" preparations, that the stimulation of respiration is relatively least marked, and that fermentation stimulation is of intermediate importance in the wound hormones, the proliferation-promoting effect being most pronounced. If these results are substantiated by more extensive investigations, the question as to whether the systemic accumulation of wound hormones, by prolonged tissue injury over long periods, can lead to a metabolic unbalance, in which the ratio of glycolysis to respiration is abnormally high, will invite investigation.

SUMMARY AND CONCLUSIONS

1. The production of wound hormones, stimulating proliferation, by ultraviolet irradiated yeast is confirmed by a more quantitative method.

2. Drying at 65° C. and autoclaving at 20 pounds for 15 minutes have no measurable effect on the potencies of the materials.

3. The yields in growth units per gram of yeast in suspension are 16 to 90 times as great in filtrates from irradiated as in those from non-irradiated suspensions under the conditions of these experiments.

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[27] Harker, G., *Australian J. Cancer Research*. In publication.

4. The evidence indicates that the wound hormones are substances released from the cells into the intercellular fluid as a physiologic response to injury rather than cellular destruction products.

5. The most potent intercellular wound hormone preparations are more potent, both on an equal weight basis and on the basis of yield in growth units per gram of yeast, than crude "bios" preparations prepared by Narayanan's method.

6. The most potent preparations have measurable activity, by the methods used, in concentrations of 8 γ per cc. (1:125,000). There is no evidence of a decrease in the relative stimulating effect at concentrations as high as 1:175.

LITERATURE CITED

Beck, W. A., *Science*, **85** (1937): 368.

Bonner, J., *Science*, **85** (1937): 183.

Bonner, J., and English, J., *J. Biol. Chem.*, **121** (1937): 791.

Calkins, G. N., *Proc. Soc. Exptl. Biol. Med.*, **9** (1912): 97.

Calkins, G. N., Bullock, F. D., and Rohdenburg, G. L., *J. Infectious Diseases*, **10** (1912): 421.

Carrell, A., *J. Am. Med. Assoc.*, **82** (1924): 255; *Proc. Inst. Med., Chicago*, **8** (1930): 62.

Cook, E. S., Kreke, C. W., and Nutini, L. G., THIS ISSUE, p. 23.

Cook, E. S., Loofbourow, J. R., and Stimson, Sr. M. Michael, O.P., Tenth International Congress of Chemistry, Rome, Italy, May, 1938, to be published in the Proceedings of the Congress.

Fardon, J. C., Norris, R. J., Loofbourow, J. R., and Ruddy, Sr. M. Veronita, O.P., *Nature*, **139** (1937): 589.

Fardon, J. C., and Ruddy, Sr. M. Veronita, O.P., THESE STUDIES, **1** (1937): 41.

Fraser, C. G., *J. Phys. Chem.*, **25** (1921): 4.

Haberlandt, G., *Beitr. allgem. Botan.*, **2** (1921): 1; *Sitz. preuss. Akad. Wiss.*, (1921): 221; *Biol. Zentr.*, **42** (1922): 145.

Harker, G., *Australian J. Cancer Research.* In publication.

Hammet, F., *Protoplasma*, **13** (1931): 331.

Loofbourow, J. R., Cook, E. S., and Stimson, Sr. M. Michael, O.P., *Nature*, **142** (1938): 573.

Loofbourow, J. R., Cueto, A. A., and Lane, Sr. M. Michaella, S.C., *Z. Zellforsch. miktoskop. Anat.* In publication.

Loofbourow, J. R., and Dwyer, Sr. Cecelia Marie, S.C., THIS ISSUE, p. 129; *Science*, 88 (1938): 191.

Loofbourow, J. R., and Morgan, Sr. M. Norbert, R.S.M., THIS ISSUE, p. 113.

Loofbourow, J. R., Schmieder, L., Stimson, Sr. M. Michael, O.P., and Dwyer, Sr. Cecelia Marie, S.C., THESE STUDIES, (1937): 79.

Lucas, G. H. W., *J. Phys. Chem.*, 28 (1924): 1180.

Narayanan, B. T., *Biochem. J.*, 24 (1930): 6.

Norris, R. J., and Hart, Sr. M. Jane, O.P., THESE STUDIES, 1 (1937): 65.

Norris, R. J., and Kreke, C. W., THESE STUDIES, 1 (1937): 137.

Norris, R. J., and Ruddy, Sr. M. Veronita, O.P., THESE STUDIES, 1 (1937): 53.

Reader V., *Biochem. J.*, 21 (1927): 901.

Reiche, H., *Z. Botan.*, 16 (1924): 241.

Richards, O. W., *Archiv. Protistenkunde*, 78 (1932): 263.

Sperti, G. S., Loofbourow, J. R., and Dwyer, Sr. Cecelia Marie, S.C., *Nature*, 140 (1937): 643; THESE STUDIES, 1 (1937): 163.

Sperti, G. S., Loofbourow, J. R., and Lane, Sr. M. Michaella, S.C., *Science*, 86 (1937): 611.

Wiesner, J., Elementarstruktur, Vienna (1892), p. 102.

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It is certain and evident to our senses that in the world some things are in motion. Now, whatever is in motion, has its motion from another, for nothing can be in motion unless it is in potentiality to that towards which it is in motion; whereas a thing moves inasmuch as it is in act, for motion is nothing else than the reduction of something from potentiality to actuality. But nothing can be reduced from potentiality to actuality except by something in a state of actuality, for example, fire, which is actually hot, makes wood, which is potentially hot, to be actually hot, and thereby moves and alters it. Now it is impossible that the same thing be at once in actuality and potentiality in the same respect, but only in different respects; for what is actually hot cannot be at the same time potentially hot, though at the same time it is potentially cold. It is therefore impossible that in the same respect and in the same way a thing should be both mover and moved, i.e., that it should move itself. Therefore, whatever is in motion must receive its motion from another. If, therefore, that from which it receives its motion is also moved, then this latter must receive its motion from another, and this in turn from another. But this cannot go on in infinitum, because then there would be no first mover, and consequently no other mover because the subsequent movers move only insofar as they receive their motion from the first mover; just as the staff moves only because it receives its motion from the hand. Therefore, we must arrive at a first mover having its motion from no other; and this everyone understands to be God.

St. Thomas Aquinas—SUMMA THEOLOGICA,
Part I, Question 2, Article 3.

(St. Thomas Aquinas died 1274).

Law 1. Every body perseveres in a state of rest, or of uniform motion in a right line, unless it is compelled to change that state by forces impressed thereon.

Law 2. The alteration of motion is ever proportional to the motive forces impressed; and is made in the direction of the right line in which that force is impressed.

Law 3. To every action there is always opposed an equal reaction: or the mutual actions of two bodies upon each other are always equal, and directed to contrary parts.

Sir Isaac Newton—MATHEMATICAL PRINCIPLES OF NATURAL PHILOSOPHY.

(Sir Isaac Newton died 1727).

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The Nihil Obstat and Imprimatur neither favor nor oppose opinions or theories here recorded. They are declarations that there is nothing in the Studies of the Institutum Divi Thomae contrary to faith or morals.

INTERCELLULAR WOUND HORMONES PRODUCED BY THE TOXIC EFFECT OF HETEROAUXIN

BY JOHN R. LOOFBOUROW AND
SISTER CECELIA MARIE DWYER, S.C.

INVESTIGATIONS in these laboratories with yeast[1] and animal tissues[2] have shown that injury of cells by ultraviolet light or other means leads to the production of growth-promoting substances by the cells as a physiologic response to injury (the "wound hormones" of Haberlandt and others[3]). Leonian and Lilly[4] have published extensive investigations of the effect of heteroauxin (β-indole acetic acid) on various fungi, algae, etc., in which they conclude that heteroauxin is a growth inhibitor rather than a growth promoter, and that when it leads to increases in growth rate in plants it may do so by its action as an irritant, inducing the plant cells to respond with the production of larger quantities of natural growth substances. In view of their investigations, it was decided to study the effect of heteroauxin on yeast, using modifications of the techniques previously employed by the authors, to determine if heteroauxin is toxic for yeast cells and, if so, whether it leads to the production of wound hormones by yeast.

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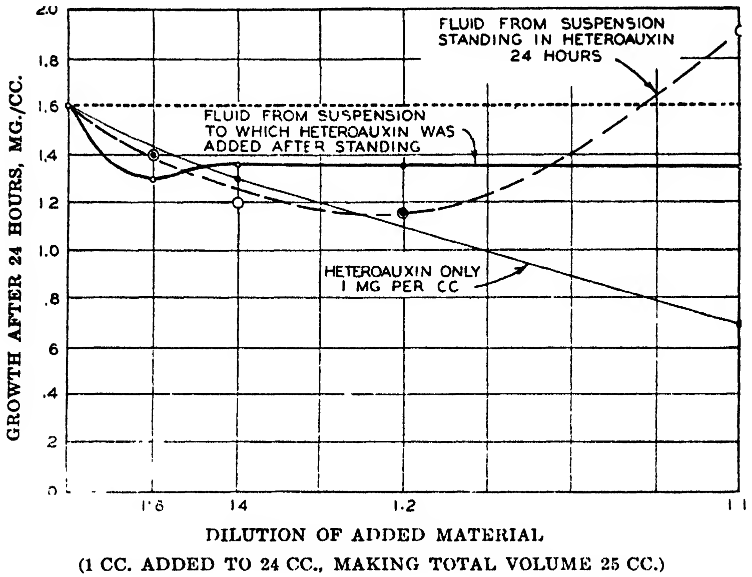
[1] Fardon, J. C., Norris, R. J., Loofbourow, J. R., and Ruddy, Sr. M. V., *Nature*, **139** (1937): 589; Norris, R. J., and Ruddy, Sr. M. V., THESE STUDIES, **1** (1937): 57; Sperti, G. S., Loofbourow, J. R., and Dwyer, Sr. C. M., *Nature*, **140** (1937): 643; THESE STUDIES, **1** (1937): 163.

[2] Sperti, G. S., Loofbourow, J. R., and Lane, Sr. M. M., *Science*, **83** (1937): 611; Loofbourow, J. R., Cueto, A. A., and Lane, Sr. M. M., *Arch. exptl. Zellforsch.*, **22** (1939): 607.

[3] Wiesner, J., Elementarstructur, Vienna, 1892, p. 102; Haberlandt, G., *Beitr. allgem. Botan.*, **2** (1921): 1; *Sitz. preuss. Akad. Wiss.*, (1921): 221; *Biol. Zentr.*, **42** (1922): 145; Reiche, H., *Z. Botan.*, **16** (1924): 241.

[4] Leonian, L. H., and Lilly, V. G., *Am. J. Botany*, **24** (1937): 135.

FIG. 1—*Typical Results of Early Experiments on Growth Potency of Filtrates from Yeast Suspensions Standing in Heteroauxin*



The first investigations were on the toxicity of heteroauxin for yeast, using as a criterion of cell injury staining with methylene blue. Fleischmann's bakers' yeast was suspended in isotonic salt solution in a concentration of 5 g./100 cc. Heteroauxin was added to such suspensions in concentrations varying from 1:1000 to 1:100,000. At approximately half-hour intervals, slides were made from these suspensions and from controls containing no heteroauxin, using methylene blue as a staining agent. In all the concentrations employed, heteroauxin caused a larger proportion of stained cells in the experimentals than in the controls. Similar evidence of the toxicity of heteroauxin for yeast was obtained by applying it to cake yeast in a concentration of approximately 1:30 in lanolin.

Experiments were then conducted to determine if heteroauxin leads to the production of wound hormones. The following procedure was used. Suspensions of yeast were made in isotonic salt solution at concentrations of either 1.75 g. or 5.0 g./100 cc. These were divided into two portions, to one of which sufficient heteroauxin was added to give a concentration of 1 mg. of heteroauxin per cc. Both suspensions were allowed to stand for 24 hours, after which heteroauxin was added to the control suspension in concentration the same as that in the test suspension, and both were centrifuged and the supernatant fluids decanted. The proliferation-promoting effect of these cell-free fluids and the effect of heteroauxin alone, were tested on yeast grown in rocker tubes in Reader's medium for 24 hours according to techniques previously described.[5] The growth after 24 hours was determined by photodensitometer readings[6] of the densities of the suspensions.

Fig. 1 is typical of the results obtained in these preliminary experiments. Heteroauxin added in quantities sufficient to lead to final concentrations in the rocker tubes of 0.04 to 0.005 mg./cc. inhibited the growth of yeast more than could be accounted for by experimental error. The results with the cell-free fluids were not definite, but there was occasional indication that the fluid from the test suspensions might contain proliferation-promoting factors.

Our previous work[5] had indicated that when the wound hormones are present in small quantities, their presence can be more conveniently detected by concentrating the solutions in a water bath or in a drying oven, neither of which procedures seems to alter the potency materially. Accordingly, fluids from suspensions prepared as described above were dried and redissolved at a concentration of 15 mg./cc. The yield was

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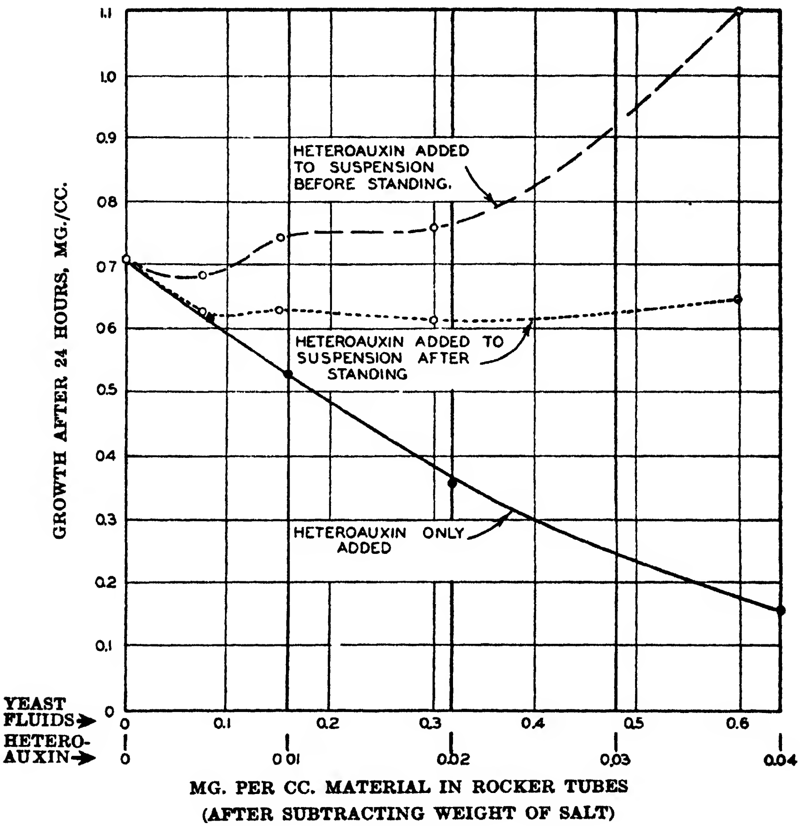
[5] Loofbourow, J. R., Dwyer, Sr. C. M., and Morgan, Sr. M. N., THESE STUDIES, 2 (1938): 137.

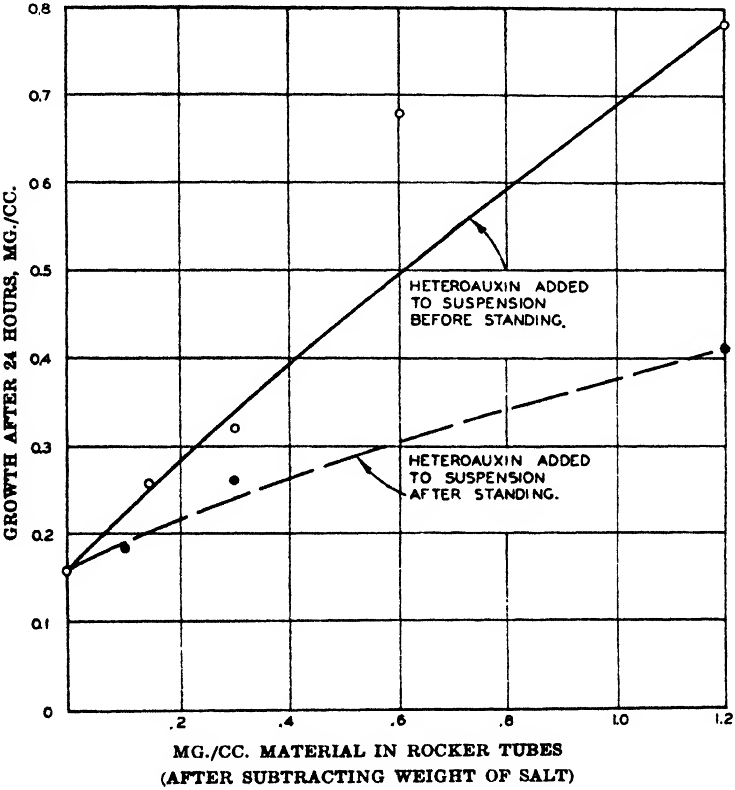
[6] Loofbourow, J. R., and Dwyer, Sr. C. M., THESE STUDIES, 2 (1938): 129.

FIG. 2—*Effect of Adding Yeast Fluids after Taking to Dryness and Making up to Higher Concentration*



somewhat greater in the test than in the control suspensions. The growth potencies of these fluids were then tested as described above. Fig. 2 shows the results of such an experiment. The marked depression of heteroauxin is evident. The fluid from yeast, to which heteroauxin was added after standing, also depressed but not as much as the heteroauxin alone, while the fluid from yeast, which had stood in the presence of heteroauxin, stimulated growth.

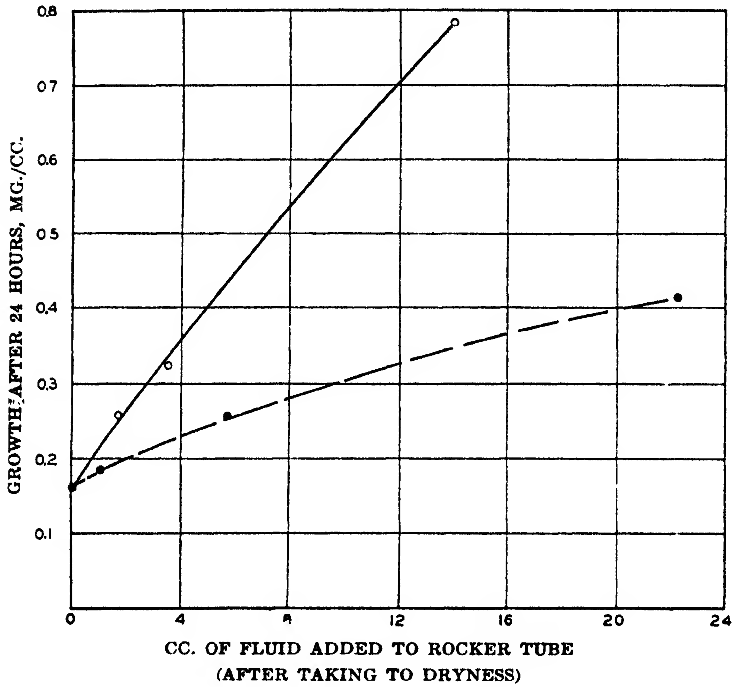
FIG. 3—*Typical Results with Materials Taken to Dryness, Made up to Higher Concentration, and Recentrifuged to Remove Undissolved Heteroauxin. Plotted on Basis of Dry Weight of Material in Fluid Added*



In all of these experiments, and in the ones to be described, samples from the test and control suspensions were periodically stained with methylene blue, and the percentage of dead cells was uniformly found to be greater in the suspensions which contained heteroauxin.

Because of the relative insolubility of heteroauxin, solutions made up after taking the fluids to dryness frequently contained

FIG. 4—*Results of Fig. 3 Plotted on Basis of Volume of Fluid Added*



a considerable amount of heteroauxin in suspension. To avoid this, the technique was modified, the solutions of the dried materials being also centrifuged, and the supernatant fluids, comparatively free from suspended matter, were used in testing the proliferation-promoting effect. In this series of experiments, the concentration of the fluids was 30 mg./cc. (not including the weight of salt). Fig. 3 is typical of the results obtained. Both the control and test fluids stimulated growth, apparently because of the lower concentration of heteroauxin present, but the test fluids were considerably more potent on an equal weight basis. As the yield was greater in the test than in the control fluids (averaging 3 to 1) the actual difference

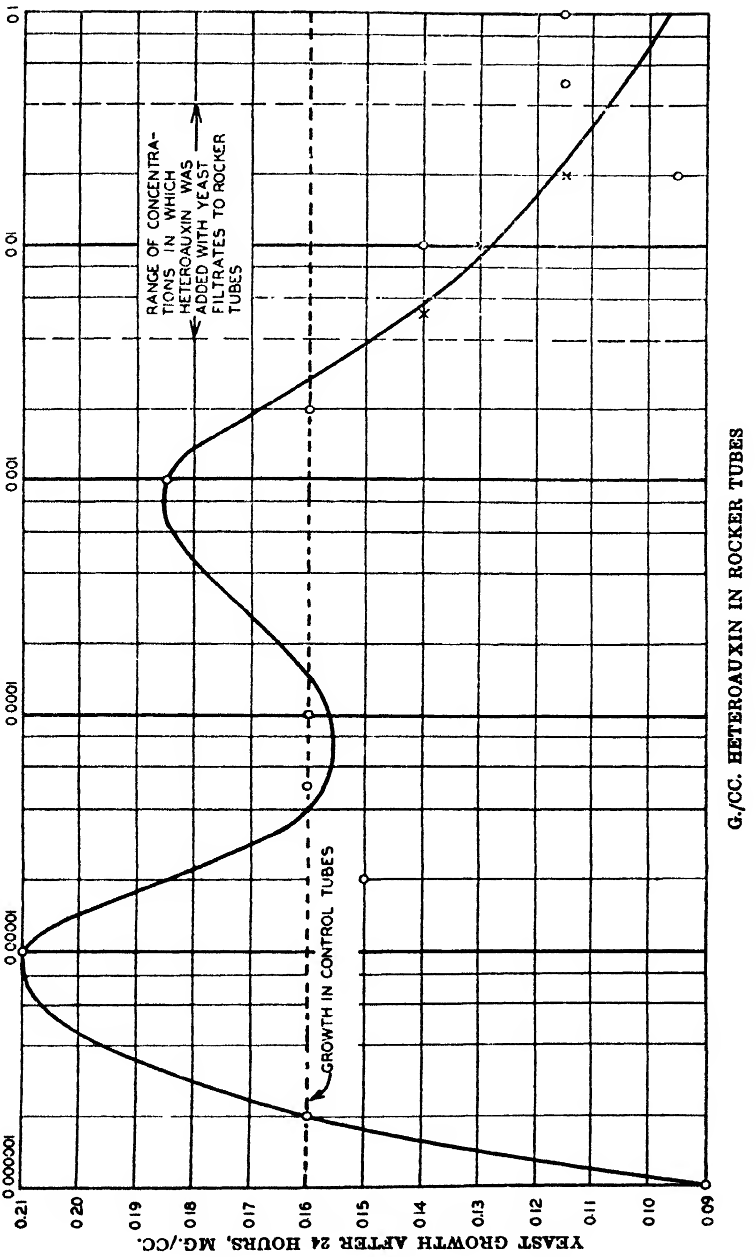


FIG. 5—*Effect of Heteroauxin only on Yeast Growth in Rocker Tubes*

in the potencies of the fluids themselves was even more marked, as illustrated by Fig. 4, plotted on an equal volume basis.

Since the introduction of heteroauxin into the rocker tubes in both the control and test suspensions could not be avoided, it was essential to determine whether this might account for the stimulating effect. It could hardly account for the difference in the effect of the control and test suspensions in an experiment such as that illustrated in Fig. 3, because in that instance the amounts of heteroauxin introduced in corresponding control and test samples were approximately the same.

To clarify this point, the effect of a wide range of concentrations of heteroauxin (from 1:10 to 1:1,000,000) was tried on the growth of yeast in rocker tubes. The results, which were not as consistent as might have been desired, are shown in Fig. 5. In the concentration range from 1:1000 to 1:100,000 there was some evidence of growth stimulation, though not to the extent obtained in the experiments with the fluids from yeast suspensions. In this range of concentrations (1:1000 to 1:100,000) stimulation by wound hormones produced by the injured cells is apparently not completely masked by the direct toxic action of the heteroauxin. The results are analogous to those obtained with other injurious agents, such as ultraviolet light, in which a certain range of dosage leads to stimulation, whereas smaller or larger doses have no stimulating effect.

In the concentration range in which the heteroauxin was introduced into the rocker tubes in the yeast fluids (greater than 1:600), only depression was noted. In this range, increased concentration leads to increased depression, which is contrary to the effects obtained with the fluids. They stimulated more markedly at higher concentrations. Thus the effect of the fluids can hardly be attributed to the heteroauxin carried with them.

In some of the experiments, microphotographs were made to determine if the stimulating effects observed were accounted for by increase in cell size rather than increase in cell number (the photodensitometer does not distinguish between the two[6]). No evidence, however, could be found for increase in cell size.

The authors believe that these experiments indicate that heteroauxin is toxic for yeast and that when used in concentration sufficiently toxic it leads to the production of proliferation-promoting factors, or wound hormones, by the yeast cells. Whether this material (or materials) is identical with the wound hormone produced by ultraviolet light, which preliminary chemical studies have shown to be somewhat nucleic acid-like,[7] remains to be determined.

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[7] Cook, E. S., Loofbourow, J. R., and Stimson, Sr. M. M., Tenth International Congress of Chemistry, Rome, Italy, May, 1938, in publication; Loofbourow, J. R., Cook, E. S., and Stimson, Sr. M. M., *Nature,* **142** (1938): 573.

LITERATURE CITED

Cook, E. S., Loofbourow, J. R., and Stimson, Sr. M. M., Tenth International Congress of Chemistry, Rome, Italy, May, 1938, in publication.

Fardon, J. C., Norris, R. J., Loofbourow, J. R., and Ruddy, Sr. M. V., *Nature,* **139** (1937): 589.

Haberlandt, G., *Beitr. allgem. Botan.,* **2** (1921): 1; *Sitz. preuss. Akad. Wiss.,* (1921): 221; *Biol. Zentr.,* **42** (1922)· 145.

Leonian, L. H., and Lilly, V. G., *Am. J. Botany,* **24** (1937): 135.

Loofbourow, J. R., Cueto, A. A., and Lane, Sr. M. M., *Arch. exptl. Zellforsch.,* **22** (1939): 607.

Loofbourow, J. R., Cook, E. S., and Stimson, Sr. M. M., *Nature,* **142** (1938): 573.

Loofbourow, J. R., and Dwyer, Sr. C. M., THESE STUDIES, **2** (1938): 129.

Loofbourow, J. R., Dwyer, Sr. C. M., and Morgan, Sr. M. N., THESE STUDIES, **2** (1938): 137.

Norris, R. J., and Ruddy, Sr. M. V., THESE STUDIES, **1** (1937): 57.

Reiche, H., *Z. Botan.,* **16** (1924): 241.

Sperti, G. S., Loofbourow, J. R., and Dwyer, Sr. C. M., *Nature,* **140** (1937): 643; THESE STUDIES, **1** (1937): 163.

Sperti, G. S., Loofbourow, J. R., and Lane, Sr. M. M., *Science,* **83** (1937): 611.

Wiesner, J., Elementarstructur, Vienna, 1892, p. 102.

Original Manuscript submitted
April 6, 1938.

A STUDY OF THE RESPIRATORY ACTIVITY OF LIVER TISSUE IN NORMAL AND VITAMIN-A-DEFICIENT RATS

By SISTER MARY VERONITA RUDDY, O.P.*

As a result of many of the author's experiments and of those reported by others,[1] which show striking variations in the respiration values of normal tissues, further information concerning the basic principles involved in tissue respiration was found necessary. When the wide range of conditions under which tissues must function and the great reserve capacity with which nature has endowed almost all tissues, is considered, it is not surprising that tissues may react to a number of environmental conditions which have not been accurately defined. Some investigators have noted wide differences in the oxygen consumption of tissue slices from the same organ not only from experiment to experiment but also within the same experiment.[2] This in a few instances has been the author's experience also.

Since a considerable number of the determinations in these laboratories were made upon the respiratory activity of liver tissue, this activity was chosen for a detailed study. It is well known that vitamin A, when taken in excess of bodily requirements, is stored in the liver. The question arose as to the effect of the vitamin-A-content upon the oxygen consumption of liver tissue. The study of rate of growth and respiratory

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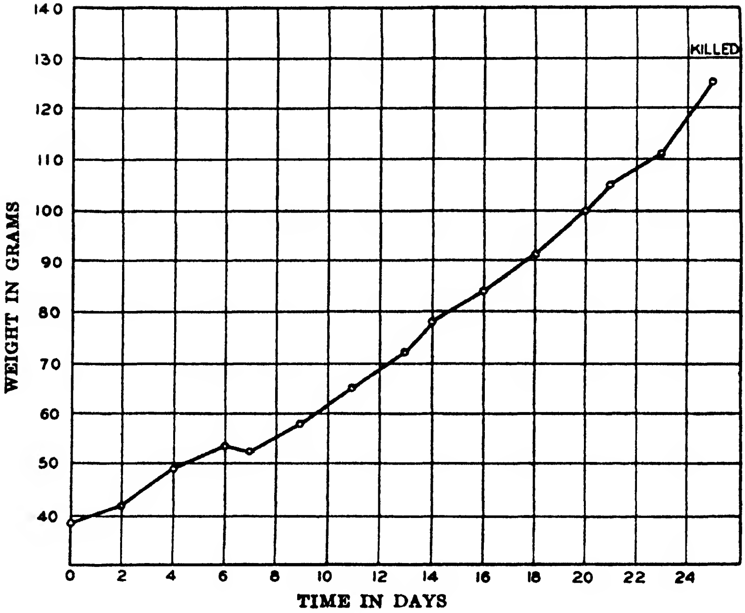
*Acknowledgment is made to Dr. Robert J. Norris for his helpful advice.

[1] Kisch, B., *Biochem. Z.*, **271** (1934): 420; **280** (1935): 55; Dixon, M., and Elliott, K., *Biochem. J.*, **23** (1929): 812; Stare, F. J., and Elvehjem, C., *Am. J. Physiol.*, **105** (1933): 655; Elliott, K., and Baker, Z., *Biochem. J.*, **29** (1935): 2433; Hawkins, J. A., *J. Gen. Physiol.*, **11** (1927-28): 645.

[2] Gemmill, C., and Holmes, E., *Biochem. J.*, **29** (1935): 338.

FIG. 1—*Growth Curve of a Normal Rat*



activity, the degree of variation which exists in animals of the same age whose rate of growth is identical, and the ratio of the weight of liver to the body weight were also the subject of this research. The results reported in this paper were obtained before the work of Chevallier and Roux[3] was reviewed by the author.

EXPERIMENTAL

In this series of experiments two groups of 25 rats each were used, all of the same age (23 days) and weighing between 35 and 40 g. They were kept under controlled conditions during the time of the experiment. One of these groups was fed the normal stock diet* supplemented by fresh vegetables (lettuce,

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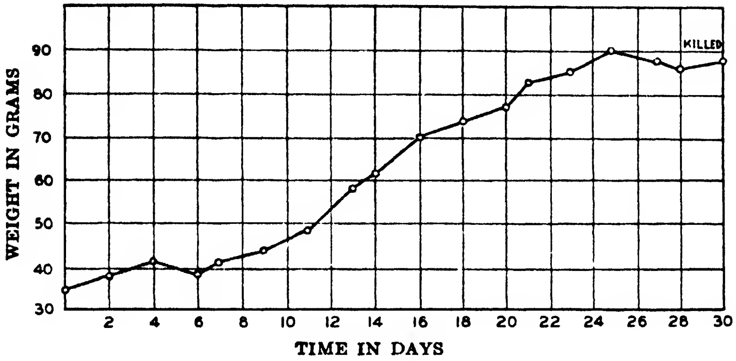
[3] Chevallier, A., and Roux, H., *Comp. rend. soc. biol.*, 118 (1935): 1348.

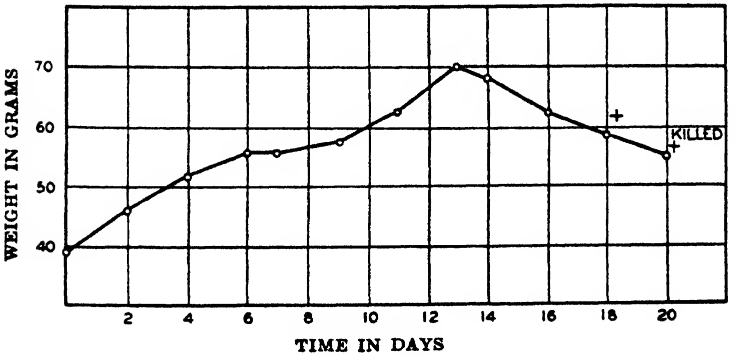
*Stock diet: Cornmeal, ground wheat, dry buttermilk, calcium carbonate and sodium chloride.

FIG. 2—*Growth Curve of a Rat Showing Cessation of Growth*



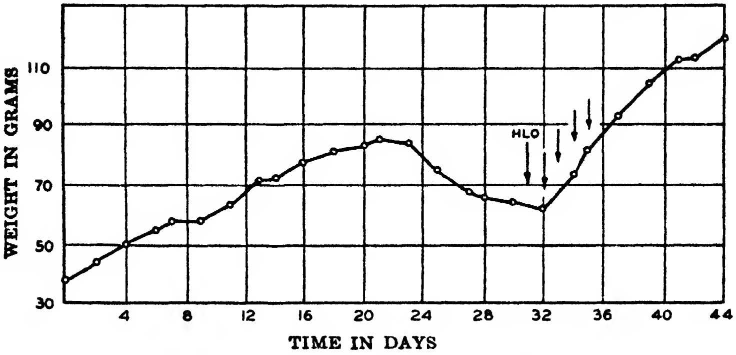
carrots and cabbage), also bread and milk. The other group was put on the vitamin-A-deficient diet as prescribed in the United States Pharmacopaeia, Eleventh Decennial Revision, 1936. The animals were weighed at the same time every second day and weight curves of the entire series recorded.

FIG. 3—*Growth Curve of a Rat Showing Vitamin A Deficiency*



+Inflammation of the Eye Lids.
Did not appear to be Typical Ophthalmia.

FIG. 4—*Growth Curve of a Vitamin-A-Deficient Rat after Administration of Halibut Liver Oil*



HLO ↓ Days on which Halibut Liver Oil was fed.

Typical curves of the different groups will be found in Fig. 1, 2, 3 and 4.

In the first series of experiments some of the animals were sacrificed as soon as the slope of the growth curve was determined, others were killed at different periods during the course of the experiment. This series of tests extended over a period of two months from April to June.

In the case of the vitamin-A-deficient rats the oxygen consumption of the liver tissue was determined at the time of vitamin depletion, cessation of growth, decline in growth and accelerated growth. This acceleration of growth was produced by the oral administration of sufficient halibut liver oils to contain approximately 1,500 U.S.P.X. units of vitamin A.

Chevallier and Roux[8] used normal rats and rats subsisting on a restricted diet and showing signs of vitamin A deficiency. They determined the oxygen consumption and the vitamin A content of the livers of these animals. The author of the present paper used the "direct method" as described by

TABLE I

Oxygen Uptake of Liver Tissues of Normal Rats

Date	Weight of Rat in g.	Weight of Liver in g.	$\frac{\text{Weight of Liver}}{\text{Weight of Rat}} \times 100$	Dry Weight of Tissue in mg.	Q_{O_2}*
April 8	38	1.3	3.4	8.3	8.6
" 8	42	1.6	3.8	10.6	9.8
" 14	47	1.8	3.8	13.9	8.8
" 14	38	1.5	3.9	12.4	9.4
" 16	56	2.0	3.6	6.1	7.4
" 16	62	2.3	3.7	8.5	7.2
" 23	93	3.5	3.8	16.1	6.9
" 23	88	3.3	3.8	14.2	8.9
" 29	96	3.4	3.5	16.6	7.8
" 29	94	3.5	3.7	14.9	10.5
" 30	110	3.9	3.5	15.1	8.6
" 30	113	4.3	3.8	12.2	7.4
May 19	129	4.3	3.3	11.8	8.3
" 19	184	5.4	2.9	10.1	7.6
" 20	174	6.8	3.3	16.0	7.4
" 22	184	5.3	2.9	7.8	8.0
" 26	224	6.5	2.9	9.9	6.4
" 31	209	6.0	2.9	10.8	9.1
					Average 8.2

*Q_{O_2} Cubic millimeters of oxygen consumed by 1 mg. of dry weight of tissue per hour.

Dixon[4] in the determination of the respiratory activity of the tissue.

In each test the animal was killed by decapitation after a fast of about 16 hours. The liver was removed and cut into slices 0.3 mm. in thickness. The slices were immediately placed in a suspension of phosphate-glucose-Ringer solution through which a stream of oxygen was kept bubbling and which was approximately 38 ° C. When a sufficient number of slices were obtained, they were separated into three equal portions and placed in three manometer flasks. These flasks had been previously charged with 3.0 cc. of Ringer solution to which had been added a suitable amount of phosphate buffer at pH 7.4 and a sufficient amount of glucose to make the concentration of the latter 0.2 per cent. The flasks were attached to the manometer, flushed with pure oxygen for about 2 minutes and then placed in the water bath. The respiration was measured over a period of 1 hour.

Chevallier and Roux do not describe their preparation of the tissue other than to state the weight and thickness used. Their determinations were made in the presence of air.

Tables I and II give the results obtained by the author. The Q_{O_2} in each case is the average of at least three determinations run simultaneously.

DISCUSSION

It will be noted from the data here presented that, although the variation from the average oxygen consumption of the normal rat liver is small and within experimental error, there is a decrement during the period of vitamin depletion and an increment when the vitamin has been restored. The variation is so slight, however, that it is questionable if the difference is significant.

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[4] Dixon, M., Manometric Methods, Cambridge, 1934.

TABLE II

Oxygen Uptake of Liver Tissue of Vitamin-A-Deficient Rats

	Date	Weight of Rat in g.	Weight of Liver in g.	$\frac{\text{Weight of Liver}}{\text{Weight of Rat}} \times 100$	Dry Weight of Tissue in mg.	Q_{O_2}	Average Q_{O_2}
Cessation of Growth	April 17	54.5	2 5	4.6	17.0	8.0	8.0
	" 18	55	2.5	4.6	17.3	8.8	
	" 21	66	2 8	4.2	14.3	9.2	
	" 22	76	3 3	4.3	12.7	6.1	
Decrease in Growth*	April 22	61	2 6	4.3	12.1	6.6	7.1
	" 25	51	2.3	4.5	11.9	7.3	
	" 28	102	4.0	3.9	6.4	7.4	
	May 6	60	1 5	2.5	11.4	7.2	
Vitamin A Restored	May 15	108	4.3	4.0	10.4	7.1	8.5
	" 21	139	5 7	4.1	8.2	10.9	
	" 23	92	3.8	4.1	9.2	7.3	
	" 29	109	3 4	3.1	10.5	7.7	
	June 4	183	6.0	3.3	8.4	10.2	
	" 5	165	5.0	3.0	8.1	7.8	
	" 9	181	7.0	3.9	10.3	8.3	

*The number of determinations run in this series is less than originally planned because of the death (caused by deficiency disease) of some of the animals which had been set apart for these experiments.

The respiration of the livers of two normal rats of the same age and having the same general growth rate was determined on the same day. The difference in the oxygen consumption varied from 2 to 36 per cent. There was no constant increase in oxygen consumption noted at any period of body growth. This is contrary to the results obtained by Peruzzi,[5] who found a rapid and persistent increase in oxygen consumption at the period in the development of the animal corresponding to a body weight of 40 to 60 g.

CONCLUSIONS

The oxygen consumption of the livers of normal rats varied from −6.4 to −10.5 and of the experimental animals from −6.1 to −10.9. The value obtained by Chevallier and Roux ranged from −6 to −7.8 for the livers of the normal rats and −6.3 to −7.5 for the livers of the depleted animals. The high oxygen uptake in our latter group was obtained from the livers of rats which had been fed halibut liver oil after vitamin A depletion. The variation of the latter group of animals was not of sufficient magnitude to conclude that vitamin A is an important factor in the respiratory activity of liver tissue although it may be one of the causes of the slight variations which have been consistently found in normal tissue.

No relation was found between the rate of growth and the respiratory activity.

The oxygen consumption of liver tissue from animals of the same age with the same general growth curves varied from 2 to 35 per cent.

The ratio of the weight of the liver to the body weight was slightly higher in the vitamin-A-deficient animals.

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[5] Peruzzi, P., *Boll. soc. ital. biol. sper.*, 10 (1935): 489.

Original Manuscript submitted
January 17, 1939.

MALT COMBINGS AS A SOURCE OF RESPIRATORY FACTORS FOR YEAST AND SKIN

By ELTON S. COOK and CORNELIUS W. KREKE

An earlier paper[1] from these laboratories reported in a non-quantitative way respiration, fermentation, and growth-stimulating activities on yeast of fractions prepared from yeast by the method of Narayanan.[2] A quantitative study[3] of fractions prepared from malt combings by the method of Lucas[4] likewise showed a division of stimulating factors among the fractions. Later the authors applied a simplified Lucas fractionation to yeast[5] and studied the effect of the fractions on the respiration of yeast, rat liver, and rat skin. It was shown that yeast contains factors which differ in their ability to increase the respiration of yeast and of animal tissues. This has been confirmed by applying adsorption techniques to the separation of the respiratory factors.[6] Thus, it has been demonstrated that both yeast and malt combings contain substances which increase the respiration of yeast, and this has been confirmed by other experiments.[7] Furthermore, yeast contains a factor which stimulates the respiration of animal tissues. A malt combings preparation has also been found to be active in stimulating the respiration of liver.[8] In

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[1] Norris, R. J., and Ruddy, Sr. M. V., These Studies, 1 (1937): 53.

[2] Narayanan, B. F., *Biochem. J.*, 24 (1930): 6.

[3] Norris, R. J., and Kreke, C. W., These Studies, 1 (1937): 137.

[4] Lucas, G. H. W., *J. Phys. Chem.*, 28 (1924): 1180.

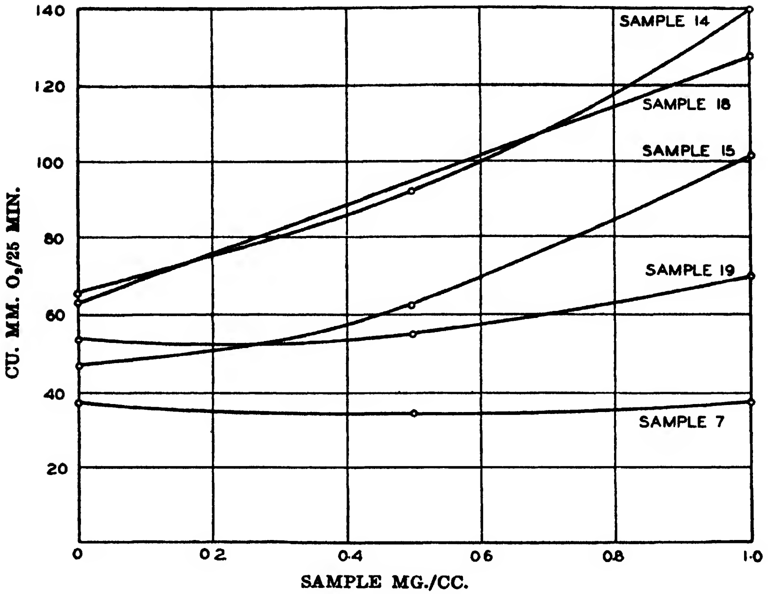
[5] Cook, E. S., Kreke, C. W., and Nutini, L., These Studies, 2 (1938): 23.

[6] Cook, E. S., and Walter, E. M., This Issue, p. 189.

[7] Cook, E. S., Hart, Sr. M. J., and Joly, R. S., *Proc. Soc. Exptl. Biol. Med.*, 38 (1938): 169.

[8] Ruddy, Sr. M. V., *Arch. exptl. Zellforsch.*, 22 (1939): 599.

Fig. 1—*Respiration Activity of Samples on Yeast*



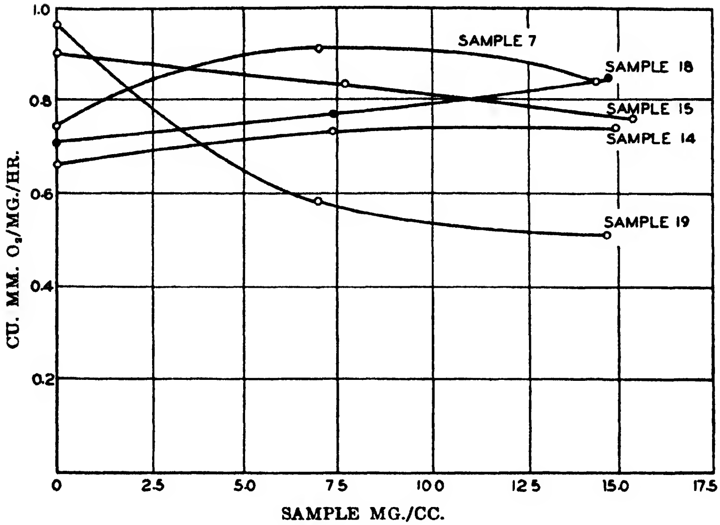
view of these facts it became of interest to investigate the comparative activities of certain available malt combings fractions on the respiration of yeast and rat skin. This is the subject of the present paper.

EXPERIMENTAL

The samples were prepared from malt combings by exactly the same procedures as previously used.[3] The sample numbers are also the same. The samples used in the present experiments were:

Sample 7 Barium hydroxide precipitate
Sample 14 Acetone precipitate from barium hydroxide filtrate
Sample 15 Acetone filtrate from barium hydroxide filtrate
Sample 18 Alcohol-insoluble, water-soluble fraction of *Sample 15*
Sample 19 Second acetone filtrate from *Sample 15*

FIG. 2—*Respiration Activity of Samples on Skin*



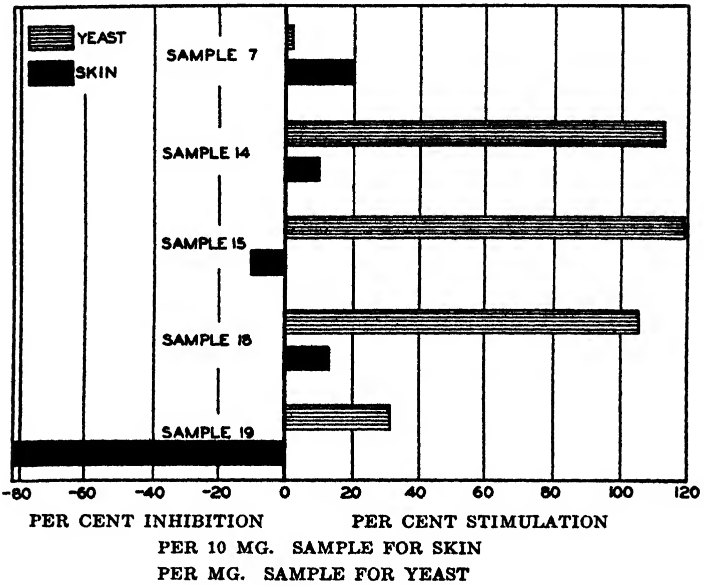
The respiration-stimulating activity of the samples was measured by the previously described techniques for yeast[4] and skin.[5] Reference should be made to the proper papers for details of the methods of preparation of samples and of the respiration techniques.

In Fig. 1 and 2 are shown the potencies of the samples in stimulating the respiration of yeast and skin, respectively. In Fig. 3 are compared the two types of activity, the potencies being expressed in percentage stimulation by 1 mg./cc. of the sample for yeast and 10 mg./cc. of the sample for skin. The stimulation refers to the following quantities of tissue and lengths of respiration: yeast at a count of 250 for a period of 25 minutes; skin, 20-40 mg. for a period of 2 hours.

DISCUSSION

The results can leave little doubt as to the non-identity of the factors which stimulate the respiration of yeast and skin.

FIG. 3—*Potency of Fractions Stimulating Respiration of Yeast and Skin*



This is especially evident in a comparison of *Samples 7, 15,* and *19.*

The two factors seem to have properties similar to those obtained from yeast.[6] For example, the skin factor is precipitated by barium hydroxide and alcohol, although, as in the case of yeast, this precipitation is not quantitative. It is also precipitated by acetone. The acetone precipitation did not give a good separation of the yeast and skin factors in the present case but this is undoubtedly due to the fact that with malt combings the precipitation was carried out rapidly. It has been found, in dealing with yeast, that the contact with acetone must be for a period of hours, or even days, to effect a complete separation. Contrary to the earlier report,[3] we find *Sample 15* to have marked yeast activity. This accords with our findings on the corresponding sample from yeast.

It is noteworthy that two of the samples (*15* and *19*) show actual inhibitory action on skin respiration, at least in the concentrations studied. This has not been encountered in the fractions from yeast although it should be pointed out that *Samples 18* and *19* have no counterpart among the yeast samples. Both of the skin-inhibitory samples stimulate yeast respiration. Although none of the fractions obtained by the Lucas fractionation of yeast inhibit skin respiration, a material obtained by steam distilling a crude yeast extract does inhibit skin respiration and increases yeast respiration when employed in the usual quantities.[9] These properties have been shown to be due to the fatty acid content of the distillate. This material at acid pH is insoluble in water and soluble in alcohol. The properties of the distillate resemble in a few respects those of the inhibitory substance from malt combings, for if skin-inhibitory *Sample 15* be precipitated with acetone, the alcohol-insoluble and water-soluble portion (*Sample 18*) possesses skin activity, but the remaining (acetone-soluble) material has increased skin-inhibition activity (*Sample 19*). (See Norris and Kreke[8] for details of these precipitations). *Sample 19*, unlike the fatty materials from yeast, is soluble in water. *Sample 15* would appear to contain both skin-inhibitory and skin-stimulatory substances, the former predominating. There is, as yet, no evidence that the skin-inhibitory fractions from malt combings are the same as the fatty acid distillate from yeast.

On comparing the activities of the fractions obtained from yeast and from malt combings it will be seen that malt combings are a poor source of the skin factor and a good source of the yeast factor.

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[9] Cook, E. S., and Kreke, C. W., THESE STUDIES, 2 (1938): 47; THIS ISSUE, p. 215; *Nature*, 142 (1938): 719.

SUMMARY

An examination of the respiratory activity on yeast and rat skin of fractions obtained by the Lucas fractionation of malt combings reveals that, as in the case of yeast, factors stimulating the respiration of yeast and skin are present. The properties of these factors seem to accord with those from yeast. In addition, malt combings appear to contain factors inhibitory to skin respiration. Malt combings are a relatively poor source of the skin factor but are an excellent source of the yeast factor.

LITERATURE CITED

Cook, E. S., Hart, Sr. M. J., and Joly, R. S., *Proc. Soc. Exptl. Biol. Med.*, 38 (1938): 169.

Cook, E. S., and Kreke, C. W., These Studies, 2 (1938): 47; This Issue, p. 215; *Nature*, 142 (1938): 719.

Cook, E. S., Kreke, C. W., and Nutini, L., These Studies, 2 (1938): 23.

Cook, E. S., and Walter, E. M., This Issue, p. 189.

Lucas, G. H. W., *J. Phys. Chem.*, 28 (1924): 1180.

Narayanan, B. F., *Biochem. J.*, 24 (1930): 6.

Norris, R. J., and Kreke, C. W., These Studies, 1 (1937): 137.

Norris, R. J., and Ruddy, Sr. M. V., These Studies, 1 (1937): 53.

Ruddy, Sr. M. V., *Arch. exptl. Zellforsch*, 22 (1939): 599.

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THE HELIANTHUS TEST

By WILLIAM A. BECK AND
SISTER MARY WINIFRED DONNELLY, R.S.M.

THE form of growth known as cell enlargement is characteristic of the higher plants. Like the increment in protoplasmic mass and cell proliferation, it must be considered a metabolic process.[1] Auxin *a* and auxin *b*, and so-called heteroauxin stimulate this form of growth.[2] The induction of cell enlargement by certain agents has served as an indicator of growth-promoting power.

There are several tests in use but the *Avena* test is the oldest and the one most frequently employed.[2c-d,3] In the *Avena* test the growth is indicated by the degree of bending of the decapitated coleoptile of an oat seedling when one side is caused to grow more rapidly than the other by means of an agent placed asymmetrically on the cut surface.

The direct measurement of growth and growth stimulation with the aid of a measuring microscope offers certain advantages over the methods usually employed. The authors used a microscope which magnified 32 times, had a range of 50 mm.

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[1] Thimann, K. V., *Ann. Rev. Biochem.*, 4 (1935): 545; *Plant Physiol.*, 13 (1938): 437.

[2] (a) Erxleben, H., *Ergeb. Physiol. biol. Chem. exptl. Pharmakol.*, 37 (1935): 186.
(b) Kögl, F., *Chem. Weekblad*, 29 (1932): 317; *Naturwissenschaften*, 21 (1933): 17.
(c) Paal, A., *Jahrb. wiss. Botan.*, 58 (1919): 406.
(d) Söding, H., *Ber. deut. botan. Ges.*, 41 (1923): 396.

[3] (a) Went, F. A. F. C., *Jaarb. Kon. Akad. Wetensch. Amsterdam*, 1927.
(b) Went, F. W., *Proc. Acad. Sci. Amsterdam*, 37 (1934): 547.
(c) Went, F. W., and Thimann, K. V., Phytohormones, Macmillan Co., New York, 1937.

and permitted the direct measurement of 0.01 mm. and a precision determination of 0.001 mm. In the other tests it was the general experience that the growth rate induced by a given agent is not constant but differs for individual plants. The cause of this variation is not altogether understood but apparently one factor is the genetic make-up of the embryo. In the *Avena* test the special strain, Victory oats (Segrehafer or Siegeshafer[3c]) (Segerhaver[4]), is usually employed. The literature shows that the age of the seed has not been taken into consideration. This is a very likely factor influencing the rate of growth. The authors used only large seeds of *Helianthus annuus*, not more than one year old and reared from a special Russian strain through three generations. The test plants were raised in individual pots filled with a washed and sterilized mixture of quartz sand and peat. A little Knop solution was added when the seeds were planted but there was no further watering during the period of the test. The seeds were planted to a depth of 5 mm. A temperature of 25° C. and a relative humidity of 95 per cent were maintained with little fluctuation.

With the above mentioned microscope it was easy to observe the enlargement of the epidermal cells of the hypocotyl, but no attempt was made to express it quantitatively for individual cells. The authors have shown by other methods that the epidermal cells in the zone 45 – 50 mm. below the base of the cotyledons are 10 times the size of those in the zone 0 – 5 mm. below the cotyledons, while the cortical cells of the lower zone were only 6 times the size of those in the upper zone.[5] The pressure produced by the epidermal cells probably prevents the further enlargement of the cortical cells, as is indicated by their higher suction tension.*

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[4] Thimann, K. V., and Lane, R. H., *Am. J. Botany*, 25 (1938): 535.

[5] Beck, W. A., Schocken, K., and Donnelly, Sr. M. W., THESE STUDIES, 2 (1938): 107.

Elsewhere the authors showed that cells farther removed than 2.5 cm. from the base of the cotyledons scarcely respond to stimulation of cell enlargement under normal conditions.[7]

In the first stage of the present work the authors determined the most favorable zone in the hypocotyl in which growth might be studied directly with the aid of the microscope. From the above it is evident that the zone is not allowed to be very long since a great difference in the growing rate of the uppermost and lowermost cells of the zone would introduce a considerable but indeterminate error in the numerical expression. By actual trial the authors found that it should not be greater than 2 cm.

The original length of the A_1-A_2 zone was 1.245 mm. The lengths of the zone in succeeding hours were as given in the table. The total growth in the hours over the original length are given in column 3. The growth in a given period in millimeters is given in column 4. The per cent growth, as given in column 5, is based on the original length of the A_1-A_2 zone because the growth is to be determined on the length of the zone as it was at the time the cells were in the original physiological state. It is the authors' purpose to express a variation in the physiology of the cells as they grow older in each succeeding period. Hence, it would be faulty to express the per cent based upon the length at the beginning of the period.

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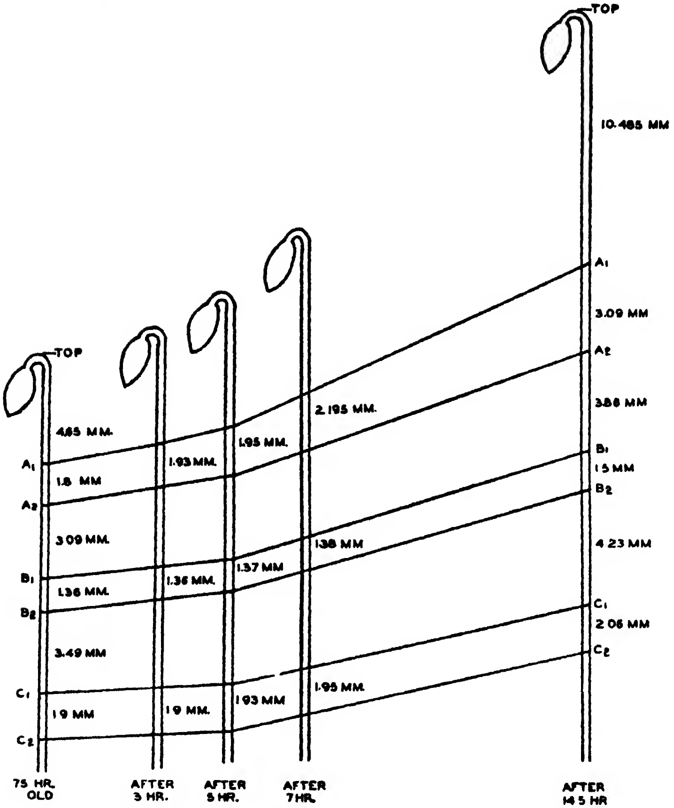
*If a section of the hypocotyl is placed in water the greater suction tension of the cortical cells causes them to take in more water than the epidermal cells so that they enlarge more,[6] causing the section to curve in such a manner that the cortical cells are on the convex side and the epidermal cells are on the concave side.

[6] Beck, W. A., *Plant Physiol.*, 3 (1928): 413.

[7] Beck, W. A., and Donnelly, Sr. M. W., THIS ISSUE, p. 259.

FIG. 1—*Diagram of the Growth in the Hypocotyl of Helianthus*


TOP
4.65 MM.
A1
1.8 MM
A2
3.09 MM.
B1
1.36 MM.
B2
3.49 MM
C1
1.9 MM
C2
1.93 MM.
1.36 MM.
1.9 MM.
1.95 MM.
1.37 MM
1.93 MM
2.195 MM.
1.38 MM
1.95 MM.
TOP
10.485 MM
A1
3.09 MM
A2
3.88 MM
B1
1.5 MM
B2
4.23 MM
C1
2.06 MM
C2
75 HR. OLD
AFTER 3 HR.
AFTER 5 HR.
AFTER 7 HR.
AFTER 14.5 HR

The results of a typical study are given graphically in Fig. 1. From this it is evident that the greatest enlargement occurs a short distance below the cotyledons. This was to be expected since the food factor[1, 8] and the pre-auxin[8c] furnished by the cotyledons are elaborated in the proliferation zone, directly below the cotyledons, to produce the necessary

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[8](a) DuBuy, H. G., and Nuernbergk, E., *Ergeb. Biol.*, 9 (1932): 358.

(b) Goebel, K., Einleitung in die experimentelle Morphologie der Pflanzen, Leipzig, 1908.

TABLE I

A Record of the Growth in the A_1-A_2 Zone in the Hypocotyl of an 80-Hour-Old Helianthus Seedling

Elapsed Time in Hours	Length in mm.	Total Growth in mm.	Growth in the Period in mm.	Per Cent Growth in the Period Based on Original Length of A_1-A_2 Zone	Per Cent Growth Per Hour
0	1.245	.....	.....	....	....
3	1.272	0.027	0.027	2.2	0.73
6	1.291	0.046	0.019	1.48	0.49
9	1.320	0.075	0.029	2.3	0.76
12	1.425	0 180	0.105	8.4	2.80
15	1.625	0.380	0.200	16.0	5.30
18	1.800	0.555	0.175	14.0	4.70
21	2.000	0.755	0.200	16.0	5.30
24	2.210	0.965	0.210	17.0	5.66
27	2.450	1.205	0.240	19.3	6.40
30	2.700	1.455	0 250	20.0	6.66
33	2 800	1 555	0 100	8.0	2.66

TABLE II

Growth in the A_1-A_2 Zone in 24 Hours of Three 80-Hour-Old Seedlings

Seedling	Length of Seedling in cm.	Length of the A_1-A_2 Zone in mm.	Time			
			First 16 Hours		Next 8 Hours	
			Per Cent Increment	Per Cent Increment per Hour	Per Cent Increment	Per Cent Increment per Hour
A	4.0	1.685	72	4.5	81.0	10.1
B	4.0	1.65	72	4.5	88.0	11.0
C	4.5	1.65	92	5.8	90.0	11.0

solutes that cause influx of water and consequent cell enlargement.[6, 9]

In 14.5 hours the uppermost zone (A_1-A_2) increased 71.8 per cent and the lowermost zone (C_1-C_2) only 21.0 per cent, and the intermediate zone (B_1-B_2) 25 per cent. The uppermost region increased 125 per cent but the increment occurred mainly after 3 hours. It was not safe to place the A_1-A_2 zone closer to the cotyledons since it would then contain cells that are too young. The most favorable time to study growth in the A_1-A_2 zone appeared to be between the seventh and the fifteenth hours after marking.

The growth rate in the A_1-A_2 zone was studied more critically. The results are given in Table I.

It became evident from the study that if the seedling is 80 hours old, about 4.5 cm. high, the zone is about 1.6 mm. long and the point A_1 is located about 6 mm. from the top, then the growth rate will be favorable and should, within reasonable limits, be the same for different plants. A series of experiments conducted at different times proved this conclusion true.

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[9] Seubert, E., *Z. Botan.*, 17 (1925): 49.

In Table II the results of experiments with three plants observed simultaneously are given, which further confirmed the authors' conclusion.

Experiments similar to the above carried out with plants 80 hours old but not 4.5 cm. high did not give the same uniform results in the growth rate of the A_1-A_2 zone. This agrees with the experience of Dijkman.[10] It is therefore recommended, in making these tests, to choose 80-hour-old plants about 4.5 cm. high.

The authors also tested the effect of the removal of the cotyledons on the growth rate in the A_1-A_2 zone. First, observations were made on test plants and controls before the removal of the cotyledons and the growth rate determined as normal in both. After the removal of the cotyledons the test plants gave evidence of inhibition of growth though cell enlargement still continued, while the growth rate of the intact control plants remained normal. This continued growth, after the removal of the cotyledons, is not surprising, since the plumule and the uppermost portion of the hypocotyl can produce auxin and because some food factor is still in the process of translocation.[11] The results suggested that test plants should have not only the cotyledons removed, but the plumule and the uppermost 5 mm. of the hypocotyl as well. Results obtained showed that under these conditions growth in the A_1-A_2 zone could be inhibited in a relatively short time.

The next step was to show that growth could be produced in the A_1-A_2 zone after inhibition, by providing growth-promoting substance to the plants stumped off in the manner described. In the experiments two kinds of control plants were used. In the one the cotyledons were retained; in the other the plants were stumped off but no growth promoter

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[10] Dijkman, M. J., *Rec. trav. botan. néerland.*, 31 (1934): 391.

[11] Overbeek, J., van, *Kon. Akad. Wetensch. Amsterdam*, 35 (1932): 1325.

TABLE III

Growth in Millimeters in the $A_1 - A_2$ Zone of Three Plants

Plant	Plants Intact		Plants Decapitated					
	Elapsed Time		Elapsed Time					
	0 Hours	16 Hours	24 Hours	40 Hours	48 Hours	64 Hours	72 Hours	88 Hours
A	1.62	3.42	3.855	3.905	3.985	4.33	4.46	5.15
B	1.595	3.465	3.90	4.59	4.89	5.51	6.07	9.48
C	1.845	3.465	No growth	No growth	No growth	No growth	No growth	No growth

A—Treated with orchid paste
B—Treated with 3-indole-acetic acid
C—Control

was supplied. The test plants were prepared as described above and growth-promoting substance was added.

In one case the growth-promoting substance was extracted from the pollinia of orchids. The pollinia were taken from the Central American species *Phragmapedulum fedemii.* To 1 g. of anhydrous lanolin was added 1 cc. of the extract, making a paste.[12] A dab of this paste was placed on the cut surface of the test plant.

In another case 1 cc. of a solution of 3-indole-acetic acid (concentration 0.2 mg. per 1000 cc. of solution) was added to 1 cc. of water and 1 g. of lanolin. It was heated to 100° C. even though 3-indole-acetic acid is not as heat stable as are auxin *a* and auxin *b*. No food factor was added in either case. The stimulation of growth in the A_1-A_2 zone was quite evident. The orchid paste remained active after very long storage in the refrigerator without special precautions.

The results of a typical experiment are given in Table III. The 3-indole-acetic acid in the given concentration stimulated more than the orchid paste, until the A_1-A_2 zone in the plant treated with acid was almost twice as long as that in the plant treated with pollinia extract.

The authors next proceeded to test the effect of a growth promoter diffusing from an agar block.[2c, 3a, 13] The heteroauxin was incorporated in a 3 per cent agar medium, in a manner similar to the preparation of the paste. The blocks 2 x 2 x 1 mm. were prepared in the usual manner.[3c] These blocks fitted neatly on the cut surface of the hypocotyls.

Very marked stimulation occurred in a much shorter period of time than was the case with the lanolin paste, probably because the growth promoter diffuses out of the agar block more readily than from the lanolin paste.

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[12] Laibach, F., *Ber. deut. botan. Ges.*, 51 (1933): 386.

[13] Thimann, K. V., and Bonner, J., *Proc. Natl. Acad. Sci.*, 18 (1932): 692.

CONCLUSIONS

If seedlings (4.5 cm. high) are raised from equally large seeds not over one year old and of the same strain, under controlled conditions (temperature 25° C. and 95 per cent relative humidity), and the cotyledons and the uppermost 5 mm. of the hypocotyl are removed, the next zone, about 1.5 mm. in length, should serve as an excellent indicator of growth stimulation. The enlargement in this zone can be determined satisfactorily and conveniently with a measuring microscope.

LITERATURE CITED

Beck, W. A., *Plant Physiol.*, 3 (1928): 413.

Beck, W. A., and Donnelly, Sr. M. W., THIS ISSUE, p. 259.

Beck, W. A., Schocken, K., and Donnelly, Sr. M. W., THESE STUDIES, 2 (1938): 107.

DuBuy, H. G., and Nuernbergk, E., *Ergeb. Biol.*, 9 (1932): 358.

Dijkman, M. J., *Rec. trav. botan. néerland.*, 31 (1934): 391.

Erxleben, H., *Ergeb. Physiol. biol. Chem. exptl. Pharmakol.*, 37 (1935): 186.

Goebel, K., Einleitung in die experimentelle Morphologie der Pflanzen, Leipzig, 1908.

Kögl, F., *Chem. Weekblad*, 29 (1932): 317; *Naturwissenschaften*, 21 (1933): 17.

Laibach, F., *Ber. deut. botan. Ges.*, 51 (1933): 386.

Overbeek, J., van, *Kon. Akad. Wetensch. Amsterdam*, 35 (1932): 1325.

Paal, A., *Jahrb. wiss. Botan.*, 58 (1919): 406.

Thimann, K. V., *Ann. Rev. Biochem.*, 4 (1935): 545; *Plant Physiol.*, 13 (1938): 437.

Thimann, K. V., and Bonner, J., *Proc. Natl. Acad. Sci.*, 18 (1932): 692.

Thimann, K. V., and Lane, R. H., *Am. J. Botany*, 25 (1938): 535.

Seubert, E., *Z. Botan.*, 17 (1925): 49.

Söding, H., *Ber. deut. botan. Ges.*, 41 (1923): 396.

Went, F. A. F. C., *Jaarb. Kon. Akad. Wetensch. Amsterdam*, 1927.

Went, F. W., *Proc. Acad. Sci. Amsterdam*, 37 (1934): 547.

Went, F. W., and Thimann, K. V., Phytohormones, Macmillan Co., New York, 1937.

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CHARCOAL AS AN ADSORBENT FOR RESPIRATORY FACTORS

BY ELTON S. COOK AND ELSIE M. WALTER

PREVIOUS studies[1] on stimulating factors in these laboratories have indicated that there are several factors which are responsible for the stimulation of the respiration and growth of yeast and animal tissue. Since the methods of separation employed in the earlier work were almost wholly chemical, it was decided to investigate adsorption methods which have been of value in the purification of many natural products. In the experiments reported here, charcoal was the only adsorbent studied. The experiments were undertaken with the following objectives in mind:

1. to find the effect of temperature on the adsorption;
2. to find the effect of water and various concentrations of alcohol as the solvent.

Further studies are in progress to determine the effect of pH variation and to examine other adsorbents.

EXPERIMENTAL

Norit charcoal was used in this series of experiments. In the first experiments the charcoal was boiled with distilled water, filtered and dried in the oven at 110° C. Later it was found that most batches of activated Norit were satisfactory without the previous treatment. (As a precaution a portion of

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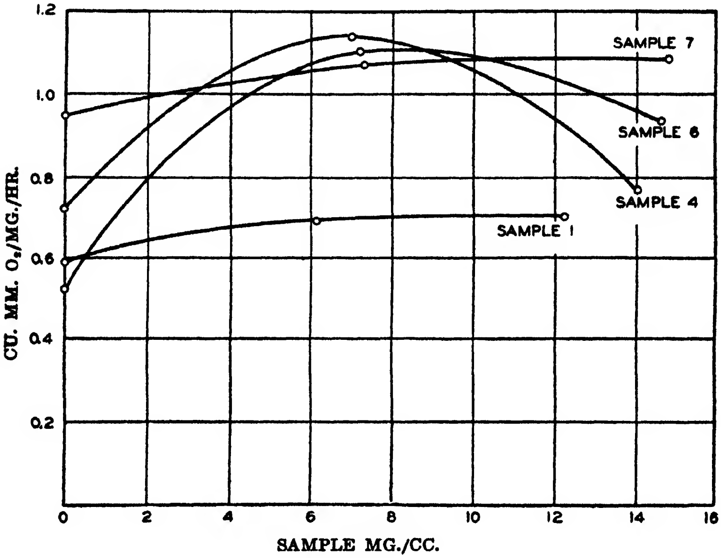
[1](a) Norris, R. J., and Ruddy, Sr. M. V., THESE STUDIES, 1 (1937): 53.
(b) Norris, R. J., and Kreke, C. W., *ibid.*, 1 (1937): 137.
(c) Cook, E. S., Kreke, C. W., and Nutini, L. G., *ibid.*, 2 (1938): 23.
(d) Cook, E. S., and Kreke, C. W., *ibid.*, 2 (1938): 47.

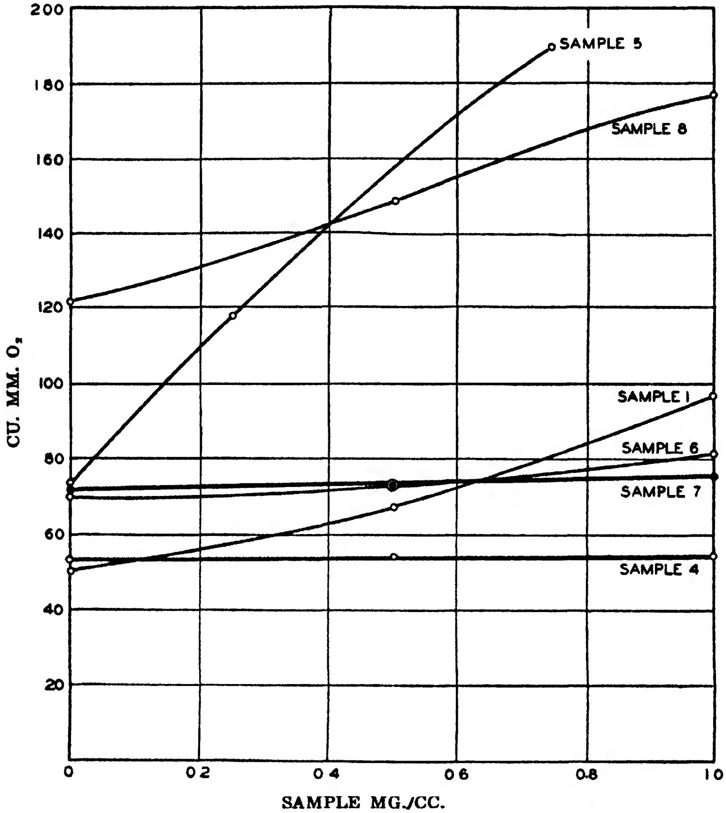
FIG. 1—*Respiratory Activity on Skin of Materials Non-adsorbed from Water*



every new batch of Norit should be tested by the above procedure.) Some experiments with the more active Darco showed that both yeast and tissue active factors were more highly adsorbed and therefore less readily separated.

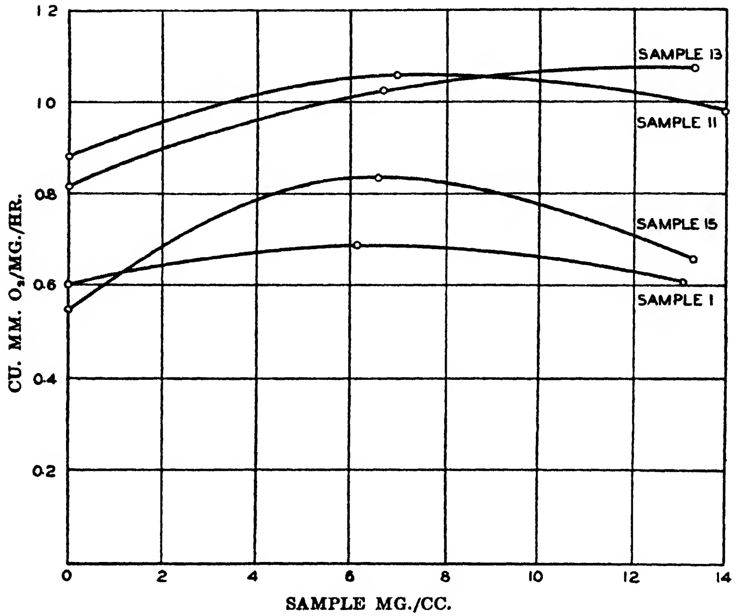
Crude respiratory stimulating factor was obtained by the previously described method[1c] of extracting Fleischmann's bakers' yeast first with 95 per cent ethyl alcohol, and then with 50 per cent alcohol at 60 – 70 ° C. for 4 hours in each case. The extracts were concentrated by vacuum distillation. The concentrate was divided into three portions and dried in the oven at 50 – 55 ° C. Portions of the dry material were dissolved in distilled water, 25 per cent ethyl alcohol, and 40 per cent ethyl alcohol to a concentration of approximately 50 mg./cc. each. It had been planned to use a 50 per cent ethyl alcohol solution, but this caused the formation of a flocculent precipi-

FIG. 2—*Respiratory Activity on Yeast of Materials Adsorbed and Non-adsorbed from Water*



tate which did not redissolve until the concentration was reduced to nearly 40 per cent. The three solutions were further divided into 3 parts to be decolorized at 5—8° C., room temperature (about 25° C.) and 60° C. The solution was either heated or cooled to the proper temperature and approximately one-tenth its weight of charcoal added. After stirring well for a few minutes, the material was filtered through a Büchner funnel. The charcoal was then removed from the

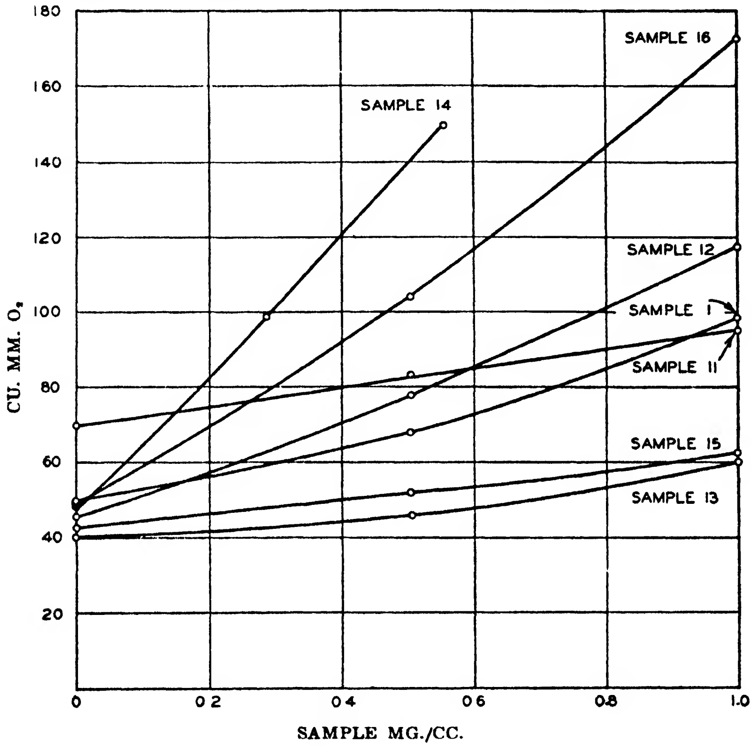
FIG. 3—*Respiratory Activity on Skin of Materials Non-adsorbed from 25 Per Cent Ethyl Alcohol*



filter, stirred in the same solvent at the same temperature as the previous treatment, and again filtered to remove adhering material. The washings were added to the original filtrate. The charcoal was again removed and eluted by stirring in distilled water at 60° C. and filtered while at that temperature. This filtrate, which should contain some of the adsorbed material, was kept separate from the original filtrate and both were dried in the oven at 50-55° C.

The original crude, after drying and making up to the proper concentration with the various solvents, was dark brown in color and had a rather disagreeable yeast-like odor. After treatment with the charcoal, in every case, the color became lighter and the odor less disagreeable. The water decolorizations were more effective in removing the color and

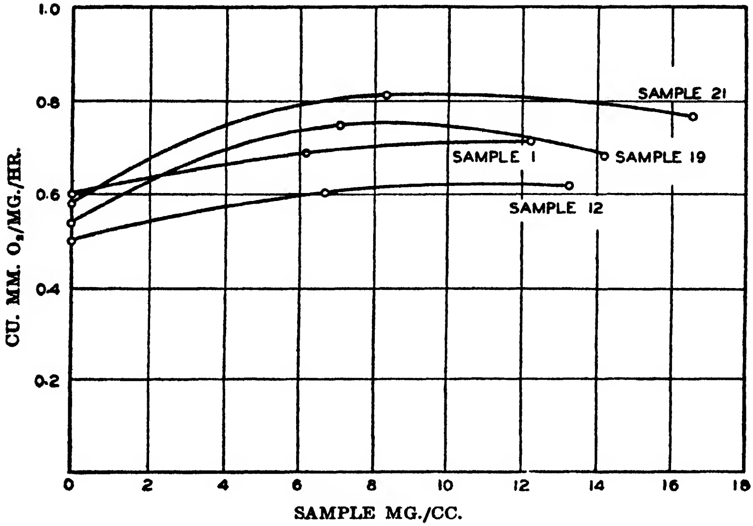
FIG. 4—*Respiratory Activity on Yeast of Materials Adsorbed and Non-adsorbed from 25 Per Cent Ethyl Alcohol*



odor than the alcoholic, and the 5-8° C. and 60° C. treatments were better than the room temperature decolorizations. Later it was found that two or three successive treatments of this water solution at 60° C. gave water-white and nearly odorless preparations.

The dried samples were made up to a concentration of 50 mg./cc. or, if the quantity of material was small, as in the case of the charcoal adsorbed material, as high a concentration as possible was used. The pH was determined by the glass electrode and adjusted to 7.3 with sodium hydroxide or

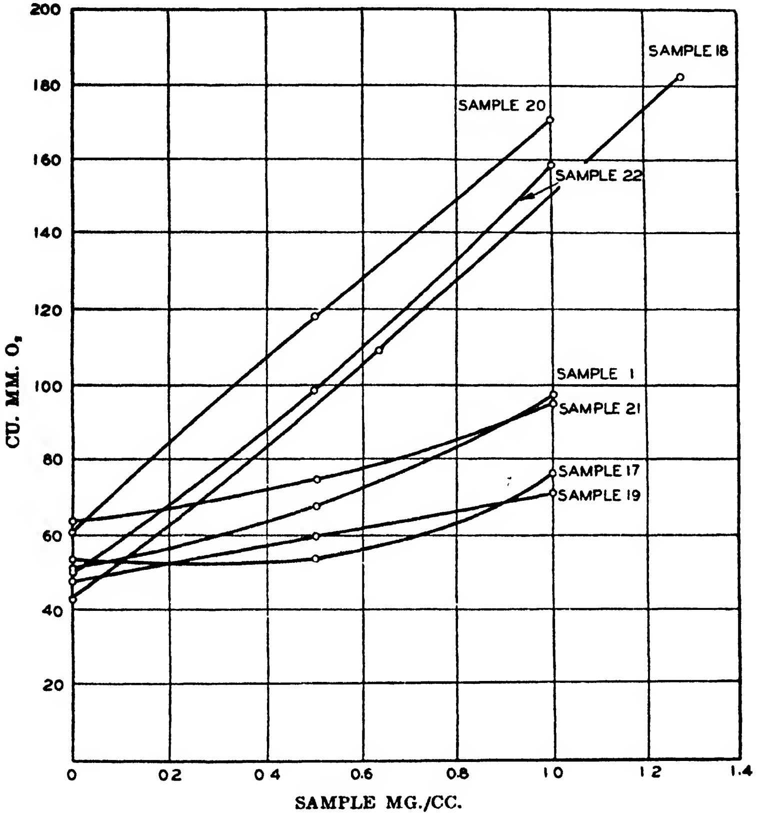
FIG. 5—*Respiratory Activity on Skin of Materials Non-adsorbed from 40 Per Cent Ethyl Alcohol*



hydrochloric acid for testing on yeast and skin respiration. If the pH of the samples was between 5.6 and 7.3 they were sterilized at 15 pounds pressure for 15 minutes before adjusting the pH. Earlier tests had shown that the respiratory stimulating factors may be sterilized within this pH range without loss of activity.[1c] Samples, the initial pH of which lay outside the above range, were adjusted to pH 7.3 before sterilization.

The decolorized, non-adsorbed materials (filtrates) were tested on both rat skin and yeast respiration, while the adsorbed material was tested only on yeast respiration, since there was insufficient material for skin experiments. The skin and yeast respiration assays were performed in the usual manner which has been described by Cook, Kreke and Nutini.[1c] All determinations were made at a temperature of 37.5° C. and a pH of 7.3. Ringer-phosphate-glucose was used as the medium. Seven manometer flasks were generally used. The

Fig. 6—***Respiratory Activity on Yeast of Materials Adsorbed and Non-adsorbed from 40 Per Cent Ethyl Alcohol***



first was used as a temperature and barometric control, the second and third as skin or yeast controls, the fourth and fifth contained the high concentration of sample (1 mg./cc. for yeast and 12 – 15 mg./cc. for skin), and the sixth and seventh were used for samples in the low concentration (0.5 mg./cc. for yeast and 6 – 7.5 mg./cc. for skin). By plotting the results the stimulation at intermediate values could be estimated. Some of the typical curves obtained are shown in Fig. 1-6.

TABLE I

Biological Activity of Samples Obtained by Charcoal Treatment

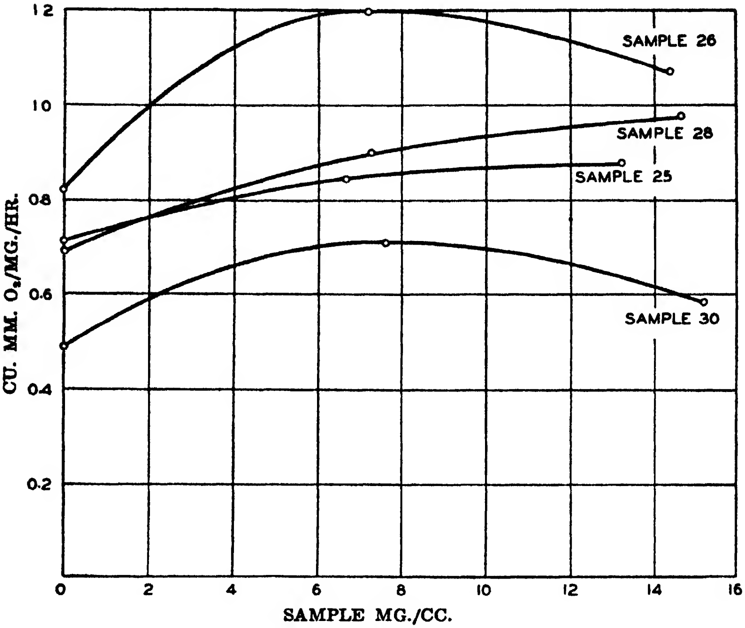
Sample	Treatment		pH before adjusting	Concentration in mg./cc.	Skin Activity		Yeast Activity	
	Solvent	Temperature			Per Cent Stimulation	Concentration in mg./cc.	Per Cent Stimulation	Concentration in mg./cc.
1. Crude	......	.....	5.60	50.0	18 11	10.0 6.55	94	1.0
4. Non-adsorbed	water	room	6.33	50.0	58 46	6.55 10.0	0	1.0
5. Adsorbed			7.40	7.82	...	.....	158	0.756
6. Non-adsorbed	water	60° C.	6.60	50.0	110 106	7.26 10.0	16	1.0
7. Non-adsorbed	water	5°—8° C.	6.65	50.0	14	10.0	6	1.0
8. Adsorbed			7.95	20.0	...	.....	46	1.0
11. Non-adsorbed	25%C_2H_5OH	room	6.15	50.0	19 17	7.0 10.0	37	1.0
12. Adsorbed			8.25	4.3	...	.....	160	1.0
13. Non-adsorbed	25%C_2H_5OH	60° C.	6.15	50.0	29 31	10.0 13.4	49	1.0
14. Adsorbed			7.92	1.75	...	.....	212	0.564
15. Non-adsorbed	25%C_2H_5OH	5°—8° C.	5.95	50.0	52 47	6.67 10.0	44	1.0
16. Adsorbed			7.85	3.54	...	.....	237	1.0
17. Non-adsorbed	40%C_2H_5OH	room	6.02	50.0	22	10.0	42	1.0
18. Adsorbed			7.86	3.97	...	.....	262	1.0
19. Non-adsorbed	40%C_2H_5OH	60° C.	6.05	50.0	35	10.0	50	1.0
20. Adsorbed			7.40	4.25	...	.....	181	1.0
21. Non-adsorbed	40%C_2H_5OH	5°—8° C.	5.95	50.0	41	10.0	53	1.0
22. Adsorbed			8.25	4.16	...	.....	253	1.0

TABLE II

Biological Activity of Miscellaneous Samples

Sample	Treatment		pH before adjusting	Concentration in mg./cc.	Skin Activity		Yeast Activity	
	Solvent	Temperature			Per Cent Stimulation	Concentration in mg./cc.	Per Cent Stimulation	Concentration in mg./cc.
23. BI Crude	......	.....	5.25	50.0	25 40	6.25 10.00	15	1.0
24. BI Non-adsorbed	40% C_2H_5OH	room	5.70	50.0	44 41	10.00 6.25	25	1.0
25. BII Crude	......	...	5.75	50.0	21 23	10.00 13.32	0	1.0
26 BII Non-adsorbed	40% C_2H_5OH then water	room 60° C.	6.05	50.0	30	14.33	13	1.0
27. BII Adsorbed	40% C_2H_5OH	room	9.05	18.33	28 20	2.20 4.39	14	1.0
28. BII Non-adsorbed	water then water	room 60° C.	6.50	50.0	36	10.00	27	1.0
29. BII Adsorbed	water	room	5.20	50.0	28 22	12.30 10.00	18	1.0
30. BII Non-adsorbed	water then twice water	room 60° C.	6.70	50.0	45 19	7.65 13.30		

FIG. 7—*Respiratory Activity on Skin of Sample 25 and Fractions Prepared from It*



The results of the biological tests are given in Table I.

Several other samples were prepared in various manners and the results of the biological tests are given in the following table (Table II). Crude samples BI and BII were prepared in the same manner as the crude of Table I but had markedly different activities. See also Fig. 7.

Ultraviolet absorption spectra were made of *Sample 6*, which showed high skin activity; *Sample 18*, which had high yeast activity; and of *Sample 1*, the crude from which *Sample 6* and *18* were made. The spectra were obtained with the Hilger quartz spectrograph with tungsten spark source and Spekker photometer. The following graph shows the results. (Fig. 8).

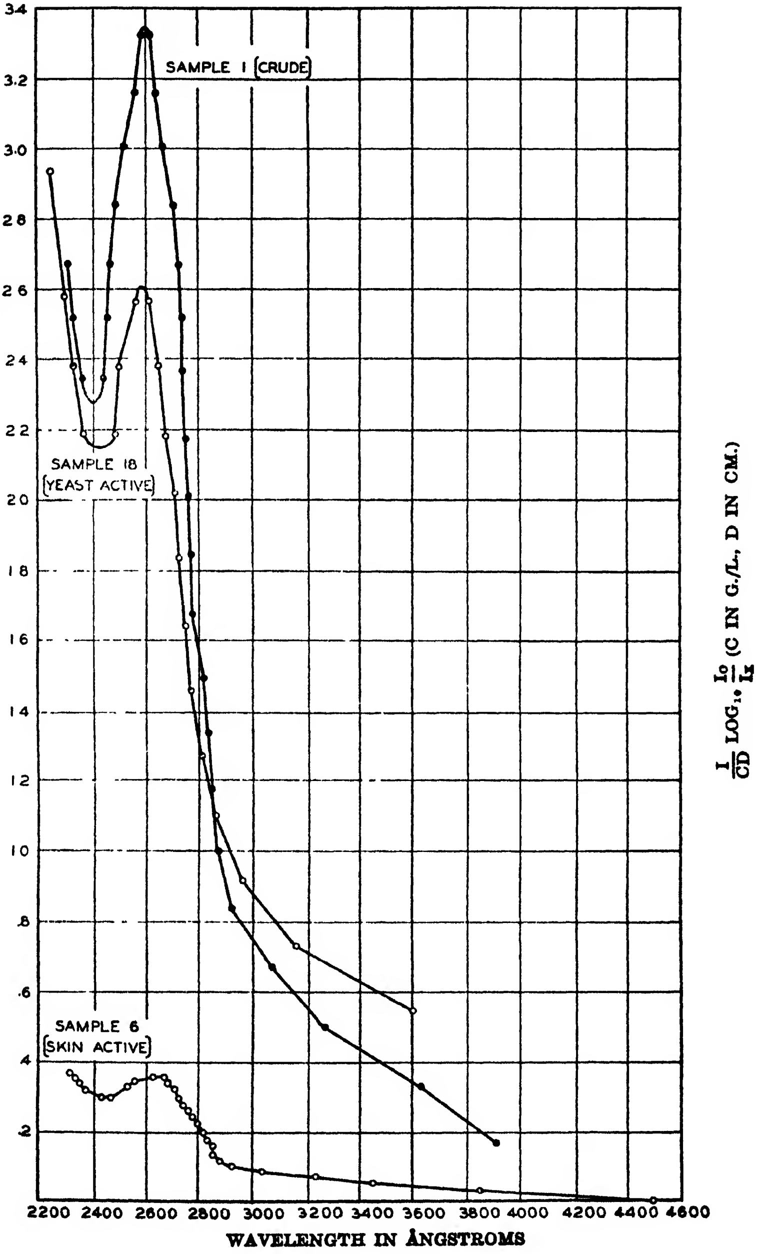


FIG. 8—*Ultraviolet Absorption Spectra of Respiratory Stimulating Factors*

DISCUSSION

An examination of the biological results of the three crudes here presented and of the results of numerous other crudes prepared in this laboratory, indicates that a high tissue activity is usually associated with a low yeast activity and vice versa. The type and degree of activity appear to vary with different batches of yeast. A similar relation of tissue and yeast activity seems also to be found in preparations from malt combings.[2]

The skin active factor is less well adsorbed by charcoal than the yeast active factor, but at the low temperature both are adsorbed in the water solution. These facts suggest that the two factors are closely related, but are not identical. The skin activity is increased most in the alcoholic solution by the low temperature adsorption and least at the room temperature adsorption in both concentrations of alcohol. Indications are similar with the yeast active fractions. However, the water solution appears more favorable for decolorizing than the alcoholic solution.

The pH of the skin active fractions was always below 7 while that of the yeast active fractions was generally above 7.

The crude preparation shows an increase in stimulation of skin respiration at the higher concentrations used in the experiments; but after adsorption, the stimulation at the higher concentration is generally either the same or less than that at the lower concentration. In most previous experiments definite decreases in stimulating power at higher concentrations have seldom been observed, perhaps due to the use of too limited a range of concentrations. However, many of the concentration curves have flattened at higher concentrations indicating that the use of concentrations above a certain amount is of no value. In impure fractions the apparent optimum concentration observed in the present experiments might be explained as due to the presence of inhibitors. Fractions inhibiting skin respiration appear to have been obtained from malt combings.[2] Evidence for an optimum concentration has been found in the effect of a steam distillate and of related pure fatty acids on yeast respiration,[1d, 3] but in this case we have shown that the inhibiting concentrations of fatty acids are toxic to the yeast cells as determined by methylene blue staining.

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[2] Cook, E. S., and Kreke, C. W., THIS ISSUE, p. 173.

The ultraviolet absorption spectra show a decrease in the absorption at 2600 Å of both the skin active and yeast active fractions as compared with that of the crude, while the biological activity has increased. It must be recalled that the very marked depression of absorption in the skin active fraction is partly due to the collection in this fraction of impurities which are presumably non-absorbing. Even so, it is impossible to correlate the respiration activity with nucleic acid-like substances as has been possible with the yeast growth-factor.[4] Work on the chemical nature of the active substances is in progress.

SUMMARY

Charcoal decolorization and adsorption were investigated on water and alcoholic solutions of the respiratory stimulating factors from yeast at various temperatures. The results indicate that the respiratory stimulating factors can be decolorized and deodorized most effectively in a water solution at 60° C.

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[3] Cook, E. S., and Kreke, C. W., *Nature*, **142** (1938): 719; Cook, E. S., and Morgan, Sr. M. N., unpublished work.

[4] Loofbourow, J. R., Schmieder, L., Stimson, Sr. M. M., and Dwyer, Sr. C. M., THESE STUDIES, **1** (1937): 79; Cook, E. S., Loofbourow, J. R., and Stimson, Sr. M. M., Tenth International Congress of Chemistry, Rome, Italy, May, 1938, *in publication*; Loofbourow, J. R., Cook, E. S., and Stimson, Sr. M. M., *Nature*, **142** (1938): 573.

The results also suggest that the yeast active and skin active factors are related but are not identical. Their adsorption by charcoal depends upon the conditions. In general, the yeast active factor is considerably more readily adsorbed than the skin factor. This fact confirms previous experiments[1] which indicate the non-identity of the two factors.

Original Manuscript submitted
December 1, 1938.

CHANGES IN RESPIRATION FOLLOWING IRRADIATION WITH X-RAYS*

BY JOHN C. FARDON, REV. WILLIAM A. SULLIVAN, O.P., REV. GUSTAVE C. BROTZGE, SISTER M. KENNETH LOEFFLER, O.P., AND SISTER MARY BASILIA ANDRUS, O.P.

MANY investigators have studied the effects of X-rays and radium on normal and malignant tissue, both *in vivo* and *in vitro*. Their work was conducted mainly to determine whether X-ray and radium radiations have a selective action upon living cells in that rapidly proliferating cells showing mitosis might be either destroyed or their division checked, while cells at rest remain unaffected. Radiations have also been employed in the study of susceptibility and resistance to transplantable tumors, and in the production of neoplasms in normal animals. Lastly, some researches have been conducted on the changes brought about by radiation on the oxidation mechanism of normal and malignant tissue.

In the earlier work in these laboratories[1] it was shown that ultraviolet radiations caused the liberation of respiratory-stimulating substances from yeast cells and a consequent increase in respiration. When radiation, however, was continued, the respiration eventually returned to normal and finally to zero. This zero value for respiration always coincided with complete killing of the suspension.

Hance and Clark,[2] in studying the division rate of paramoecia after X-raying, observed a slight initial depression in division which lasted from 2 to 5 days after irradiation. This depression was followed by complete recovery whenever the X-ray dose was small. X-raying for a period of 3 to 4 hours produced opposite results. Prime[3] found that radium in sufficiently large doses so injures the nucleus of the cell growing *in vitro* as to prevent further mitosis. This injury to the mitotic power of the cell does not, however, prevent a marked increase in the area of the culture due to migration of cells. The same effect was observed by Jones and Mottram[4] on carcinoma and sarcoma *in vitro*. Prime also found that when there is a marked outwandering of cells after radiumization, but no mitosis, the tissue would not grow when inoculated into mice. The continuance of outgrowth after X-raying was found also by Kimura.[5] Mitotic figures were limited to a minimum after an exposure of E.8. After exposure of E.12, however, mitosis was no longer observed, and the treated tissue produced no tumor when inoculated into mice. Love,[6] working with the mouse carcinoma *in vivo*, found that after an X-ray dose of 250 r units, 77 mitotic figures were observed per 200 fields. At 500 r units there remained 51 division figures. The number of control mitotic figures per 200 fields was found to be 233.

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*Research conducted under the Sir Charles F. Williams Fellowship.

[1] Fardon, J. C., Carroll, Sr. M. J., and Ruddy, Sr. M. V., THESE STUDIES, 1 (1937): 17.

[2] Hance, R. T., and Clark, H., *J. Exptl. Med.*, 43 (1926): 61.

In these laboratories (unpublished results) it has been found that mitosis of cells growing *in vitro* can be checked by short exposures to ultraviolet. Cells exposed during the metaphase of division have been observed to return to the resting stage without completing the division. The amount of irradiation given these cultures was kept below the lethal dose. A series of preliminary experiments also indicated that tissues cultured *in vitro*, but failing to grow, could be induced to proliferate after exposure to certain doses of X-rays.

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[3] Prime, F., *J. Cancer Research*, 2 (1917): 107.

[4] Jones, C. P., and Mottram, J. C., *Arch. Middlesex Hosp.*, 23 (1914): 21.

[5] Kimura, N., *J. Cancer Research*, 4 (1919): 95.

[6] Love, W. H., *Med. J. Australia*, 2 (1933): 70.

Murphy et al.,[7] investigating the effect of X-rays on cancer immunity, have observed that a marked lymphocytosis arises after cancer inoculation in animals both with natural and induced immunity. When this lymphocytosis is prevented by previous exposures to X-rays, the resistant state is lost. After extensive depletion of lymphocytes by X-rays active regeneration of the lymphocytes is soon initiated and continues to over-production. These investigators further demonstrated that a direct effect of the X-rays on the animal was to render it more highly resistant to replants of its own cancer than would normally be the case. Tumor grafts taken from the host and X-rayed and then returned, grew as well as the controls.

When erythema of the skin was produced by X-rays and a week later a cancer graft was inoculated at the erythema area, the percentage of takes was lower than when the graft was inoculated at another site. Mice treated with small doses of X-rays and inoculated with cancer immediately afterward, show a marked suppression of lymphoid proliferation. If, however, the cancer inoculation is made seven days after the exposure to X-rays, when the primary lymphoid stimulation, known to occur soon after the X-ray treatment, has begun, a second stimulation takes place in a large proportion of mice thus inoculated. Changes in the blood of mice, which have been X-rayed and inoculated with cancer seven days afterward, show that the state of resistance to cancer inoculation is associated with blood lymphocytosis, as is the case in all other varieties of immunity to transplantable cancer so far studied.

The effect of X-rays and radium on the respiration of malignant tissue has also been investigated by several observers. Löw-Beer and Reiss,[8] for instance, found that after X-raying the Jensen rat sarcoma, a maximum reduction of oxidation takes place on the second or third day following the treatment. The same observation was made by Crabtree[9] on both tumor and normal tissue after exposure to radium rays. Franks and Shaw[10] claim that radiation primarily inhibits respiration.

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[7] Murphy, J. B., and Morton, J. J., *J. Exptl. Med.*, 22 (1915): 204, 800; Murphy, J. B., and Taylor, D., *ibid.*, 28 (1918): 1; Murphy, J. B., Hussey, G., Nakahara, W., and Sturm, E., *ibid.*, 33 (1921): 299; Nakahara, W., and Murphy, J. B., *ibid.*, 33 (1921): 429, 433; Murphy, J. B., Maisin, J., and Sturm, E., *ibid.*, 38 (1923): 645.

In these laboratories the radiation experiments were in part inspired by the work of Warburg,[11] who found a lowered respiration and an active glycolysis in tumor tissue. Warburg advanced the hypothesis that tumor production might result from an oxygen deficiency of normal tissue. He supposes that in normal resting tissue there exists an uneven distribution of glycolytic activity, i.e., very few cells glycolyze strongly while the greater number of cells do not glycolyze at all. Should such a mixture of cells suffer an oxygen deficiency through pressure, sclerosis, bacteria, etc., then the cells unable to glycolyze must die. The authors' plan was to irradiate a series of mice with X-rays, and at various intervals after irradiation, measure the change in respiration of the area treated, as compared with the respiration of the unirradiated tissue of the same animal. By following this procedure the authors anticipated a depression in respiration of the treated tissue as a precursor to a visible change to malignancy. The experiments thus outlined were also to indicate whether lowered respiration with a consequent change in the glycolytic ratio was a product of active neoplastic proliferation, or whether the disturbance in metabolism initiated the unorganized growth of tissue.

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[8] Löw-Beer, A., and Reiss, M., *Strahlentherapie*, 42 (1931): 157.

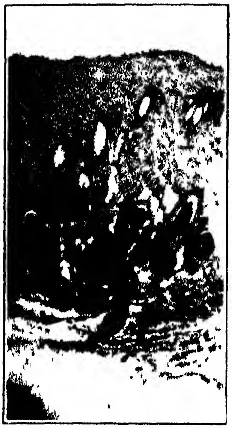
[9] Crabtree, H., Tenth Scientific Report Imperial Cancer Research Fund, London, (1932), p. 71.

[10] Franks, W. R., and Shaw, M. M., *Am. J. Cancer*, 22 (1934): 601.

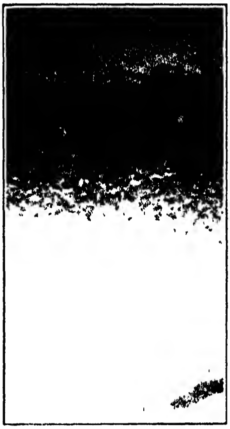
[11] Warburg, O., *J. Cancer Research*, 9 (1925): 148.

PLATE I

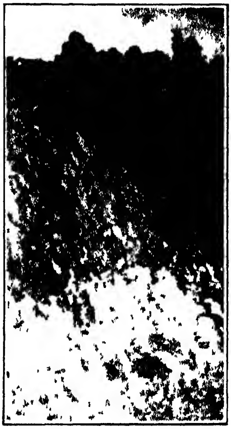
Histological Sections of X-Rayed Skin



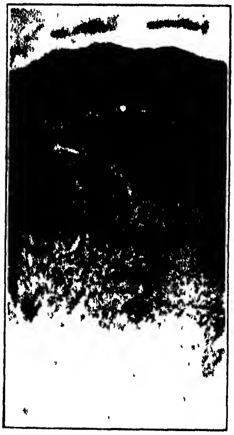
Control



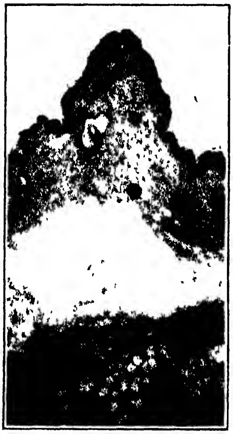
5-Day



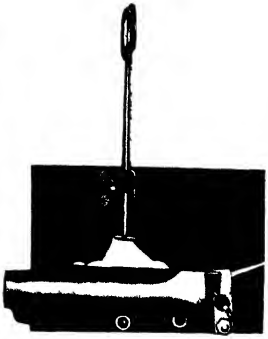
16-Day



32-Day



50-Day



Mouse Holder

Since the respiration of tissue from locally irradiated mice was measured immediately after treatment as well as at intervals extending over a period of about seven weeks, an interesting observation was made, which, though not quite satisfying the original purpose of the experiment, nevertheless, was thought to make the present report justifiable. Upon consideration of the results obtained, it became apparent that there also existed a correlation between this change in respiration and the X-ray-induced immunity experiments of Murphy and his coworkers.[7]

EXPERIMENTAL

Sixteen mice were given a dose of X-rays at 45 kv. and 10 Ma. for a period of 25 minutes, and with a target distance of 20 cm. An area of about 3 sq. cm. of skin previously shaven was exposed to irradiation. The mouse under treatment was held in a lead-lined protecting tube containing a slot, through which a portion of the skin was pulled and held in place with a clamp, as illustrated in Plate I.

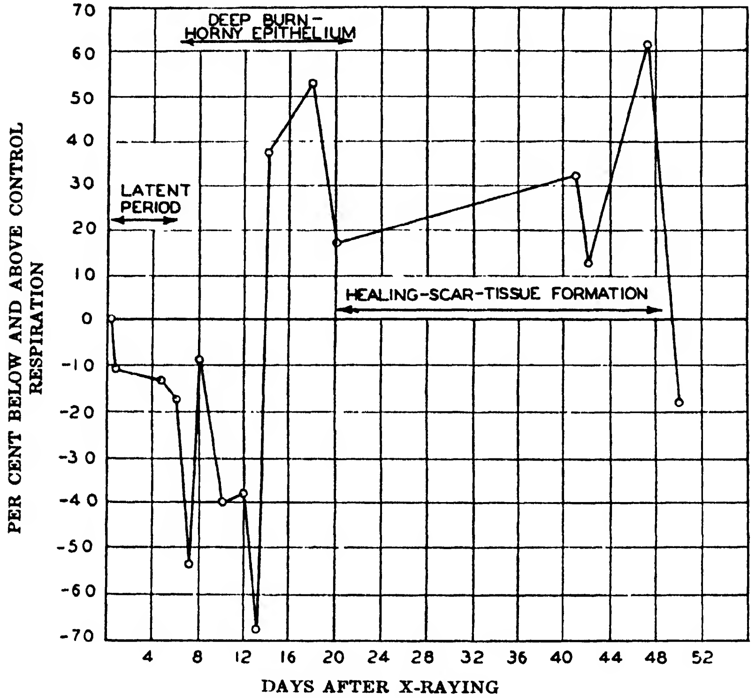
For the first experiment a mouse was chosen 3 hours after irradiation. The animal was sacrificed by decapitation, and the X-rayed area of skin carefully removed and freed of adipose and connective tissue. The small clamped area, protected from the radiation, and possibly injured by pressure from the clamp, was cut from this piece and discarded. The remaining tissue was cut into small blocks about 1 mm. square. An equal area of skin, removed from the opposite side of the mouse, was used as control. The experimental and control portions of skin were each divided into 2 equal parts and placed into 4 Warburg-Barcroft manometer flasks each containing 3 cc. of Ringer solution in the outer well and 0.3 cc. of a 5 per cent KOH solution in the inner well. After a 15-minute

TABLE I

Effect of X-rays upon the Respiration of Mouse Skin

Experiment	Time after X-raying in days	QO_2 of Control			QO_2 of Experimental			Per cent Stimulation	Sex	Age of Mouse in Months	Condition of Irradiated Area
		Flask No. 1	Flask No. 2	Average	Flask No. 3	Flask No. 4	Average				
1	3/24	.888	.937	.913	.935	.886	.911	− 0.2	♂	4	normal
2	17/24	.647	.432	.540	.389	.566	.477	−11	—	–	normal
3	5	.433	.494	.463	.387	.418	.403	−13	♂	4	sloughed
4	6	.835	.842	.839	.736	.639	.687	−18	—	–	deep burn
5	7	.580	1.328	.954	.571	.329	.450	−53	♂	3	———
6	8	.925	.726	.825	.685	.822	.753	− 9	♀	–	———
7	10	.490	.789	.640	.370	.405	.387	−40	♂	3	———
8	12	.935	.807	.871	.506	581	.544	−38	♂	4	sloughed
9	13	.070	106	.088	.019	041	.030	−68	♂	3	horny epithelium
10	14	.791	.839	.815	1.072	1.166	1.119	+38	♀	3	sloughed-healing
11	18	.514	.446	.480	.728	.741	.734	+53	—	5	burn
12	20	.447	485	.466	.543	.551	.547	+17	♂	4	partial healing
13	41	.700	.682	.693	.886	.945	.915	+32	♂	3	cicatrized
14	42	.850	.712	.781	.829	.941	.885	+13	♂	3	cicatrized
15	47	.563	.545	.554	.976	.823	.899	+62	♂	4	cicatrized
16	50	1.461	1.406	1.434	1.168	1.187	1.178	−18	♀	–	cicatrized

FIG. 1—*The Effects of X-ray Burns on the Respiration of Mouse Skin*



equilibrium period the respirometers were closed to the outside air and respiration measurements taken after 2 hours. The tissue was then carefully removed from the manometer flasks and dried overnight in a drying oven at 110° C. Corrections were made for the dry weights of the tissue. This procedure was followed throughout the entire series of experiments.

The results of all the respiration experiments are tabulated in Table I, which gives the time after X-raying, the sex and age of the mice, the condition of the treated area of skin at the time of respiration determination, and the Q_{O_2} of the experimental and control skin.

DISCUSSION

There is considerable variation in the Q_{O_2} of the animals used, which could be correlated with neither age nor sex; this, however, is to be expected as experiments in these and other laboratories have given ample evidence of great variation in respiration rate between individual animals. In Flasks No. 1 and 2 are portions of control tissues, and in Flasks No. 3 and 4 portions of the irradiated tissues. It is at once apparent from the curve shown in Fig. 1 that soon after irradiation the respiration of the skin begins to drop and continues to do so until about 13 days after X-raying. About this time the appearance of the treated skin changes from normal to a deep burn accompanied by sloughing, and with a very rapid rise in respiration bringing the oxygen consumption of the treated skin considerably above that of the control skin. It was thought that the rawness of the wound in evidence at this time might be accompanied by bacterial contamination, which produced this sudden increase in oxygen consumption. However, upon making smears from both the control and experimental areas of skin it was found that, though some organisms were present, they were not numerous, nor did more appear in the burned area than in the control.

From the 14th day after irradiation to about the 47th day a consistent stimulation of respiration was noted. Throughout this period of greater oxygen consumption the appearance of the skin changed from a sloughing and horny epithelium to complete cicatrization. The last point on the curve shows a depression in respiration of 18 per cent. Observations beyond 50 days have not been made at this writing. It is felt, however, that some minor fluctuations will persist until the treated portion of tissue is restored to normal metabolism. Should a malignant condition arise as a result of irradiation, a continued or a second decline in respiration, either prior to proliferation or concurrent with it, might be expected.

The histological pictures of the X-rayed areas of skin taken at various intervals after irradiation do not reveal anything of special significance. It will be observed in Plate I, in which a control, a 5-day, a 16-day, a 32-day and a 50-day section of stained skin are shown, that all the experimental sections present quite a different picture from that of the control. There is an apparent degeneration of the hair follicles in all the treated areas. Maximum lymphoid tissue infiltration appears between the 16 and 32-day sections, the latter showing a considerable thickening of the *rete mucosum* and very extensive invasion of lymphoid cells into the *cutis vera.* It is to be noted that maximum lymphoid infiltration occurs during the period in which the respiration of the irradiated skin is stimulated, but does not continue as long as the stimulation period.

Though the general observations made by other investigators reveal a depression in respiration after irradiation with X-rays, these experiments show that the initial inhibition of respiration is followed by a definite period of stimulation. The importance of extending the experiments over a long period of time is well emphasized. Definite conclusions concerning the cause of this reversal in oxygen consumption cannot be legitimately drawn at this time, but several tentative explanations might safely be ventured:

1. A sufficiently large dose of X-rays will naturally be followed by a period during which many cells are dying, thus causing a decrease in the oxygen consumption of the affected tissue. The period of destruction is followed by a period of repair, and the accompanying stimulation of the metabolic processes overshoots the mark, resulting in a consequent higher oxygen up-take.

2. Following the period of depressed respiration due to cellular destruction, there appears besides tissue proliferation an active regeneration of lymphocytes, which, according to Murphy, continues to over-production. This over-production of lymphoid tissue in the treated area might readily be responsible for the increase in oxygen consumption.

From the information so far obtained concerning the two phases of respiration activity after X-raying, it is interesting to note how the researches of Murphy on cellular reaction have a tendency to fit into this pattern, but one must be cautious in drawing definite conclusions, for the X-ray dosages used by Murphy were considerably smaller than those used in the authors' experiments.

Murphy found a low percentage of tumor-takes when the transplants were made intracutaneously one week after subjecting a local area of skin to an erythema dose of X-rays. When the graft was made subcutaneously it grew equally well in both the irradiated and protected areas. Murphy found, furthermore, that the skin layers were markedly infiltrated by lymphoid cells several days after the X-raying, but this reaction did not extend deeper than these layers. It is to this local lymphoid reaction produced by X-rays that Murphy attributes the immunity. By giving repeated small doses of X-rays over 7 successive days, Murphy was able to increase the number of tumor-takes in mice previously immunized. This partial lowering of immunity he claims to be due to a destruction of a large part of the circulating lymphocytes.

From the experiments conducted in these laboratories and graphically presented in the curve of Fig. 1, it will be seen that a decided depression in respiration occurs about one week after X-raying and the respiration continues to drop until after the second week. This period might well be analogous to Murphy's experiment in which, after 7 days of successive

X-raying, the immunity was greatly impaired due to the absence of lymphoid tissue in the treated area. The period of stimulation of respiration, on the other hand, might find its parallel in the experiment in which Murphy gave but a single erythema dose to the skin and found a subsequent low percentage of tumor-takes during marked lymphocytosis.

It will be of further interest to determine the degree of immunity in the X-rayed area of skin after the initial depression of respiration and during the period of stimulation. It is yet to be determined whether the initial period of oxygen depression is entirely due to degeneration and dying of cells after irradiation, and the subsequent period of stimulation due to an accumulation of lymphocytes and new tissue cells, or, whether these are due to a more direct interference with the respiratory mechanism of the cells.

SUMMARY

1. A period of depressed respiration follows a dose of X-rays sufficient to produce a burn on a localized area of mouse skin.
2. The period of depression (about 13 days) is followed by a period of stimulation, which continues for some 35 days.

LITERATURE CITED

Crabtree, H., Tenth Scientific Report Imperial Cancer Research Fund, London, (1932), p. 71.

Fardon, J. C., Carroll, Sr. M. J., and Ruddy, Sr. M. V., THESE STUDIES, 1 (1937): 17.

Franks, W. R., and Shaw, M. M., *Am. J. Cancer*, **22** (1934): 601.

Hance, R. T., and Clark, H., *J. Exptl. Med.*, **43** (1926): 61.

Jones, C. P., and Mottram, J. C., *Arch. Middlesex Hosp.*, **23** (1914): 21.

Kimura, N., *J. Cancer Research*, **4** (1919): 95.

Love, W. H., *Med. J. Australia*, **2** (1933): 70.

Löw-Beer, A., and Reiss, M., *Strahlentherapie*, **42** (1931): 157.

Murphy, J. B., and Morton, J. J., *J. Exptl. Med.*, 22 (1915): 204, 800.

Murphy, J. B., and Taylor, D., *ibid.*, 28 (1918): 1.

Murphy, J. B., Hussey, G., Nakahara, W., and Sturm, E., *ibid.*, 33 (1921): 299.

Murphy, J. B., Maisin, J., and Sturm, E., *ibid.*, 38 (1923): 645.

Nakahara, W., and Murphy, J. B., *ibid.*, 33 (1921): 429, 433.

Prime, F., *J. Cancer Research*, 2 (1917): 107.

Warburg, O., *J. Cancer Research*, 9 (1925): 148.

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December 10, 1938.

RESPIRATORY ACTIVITY OF A STEAM DISTILLATE FROM YEAST

BY ELTON S. COOK AND CORNELIUS W. KREKE

In an earlier note[1] the authors described two preparations of a material obtained by the steam distillation of an aqueous-alcohol extract of yeast. These samples of steam distillate, referred to as *A* and *B*, were worked up essentially by conversion to the water-soluble potassium salt. A description of these fractions and details of the experimental procedures employed in their preparation were given in the paper cited.

A third preparation has also been made by a method similar to *B* but this was obtained from the aqueous distillate (13 liters) by extraction with ether. After drying the ether solution over sodium sulfate, removal of the ether left an oil containing some solid. This is *Sample C*. It is evident that the ether extracted somewhat more material than was obtained in the earlier experiments. This material, which had an odor suggestive of isovaleric acid, gave an iodine number of 5.5 by the Hanus method, and gave negative tests for nitrogen, sulfur, halogens and sterols (Liebermann-Burchard). These properties, together with solubility in the common fat solvents and in alkali, are strongly suggestive of a mixture containing saturated fatty acids.[2]

These three preparations were tested for respiratory activity on yeast and rat skin by the previously described "direct" Warburg manometric techniques[3] using Ringer-phosphate-glucose (0.02 per cent glucose) as the suspending medium.

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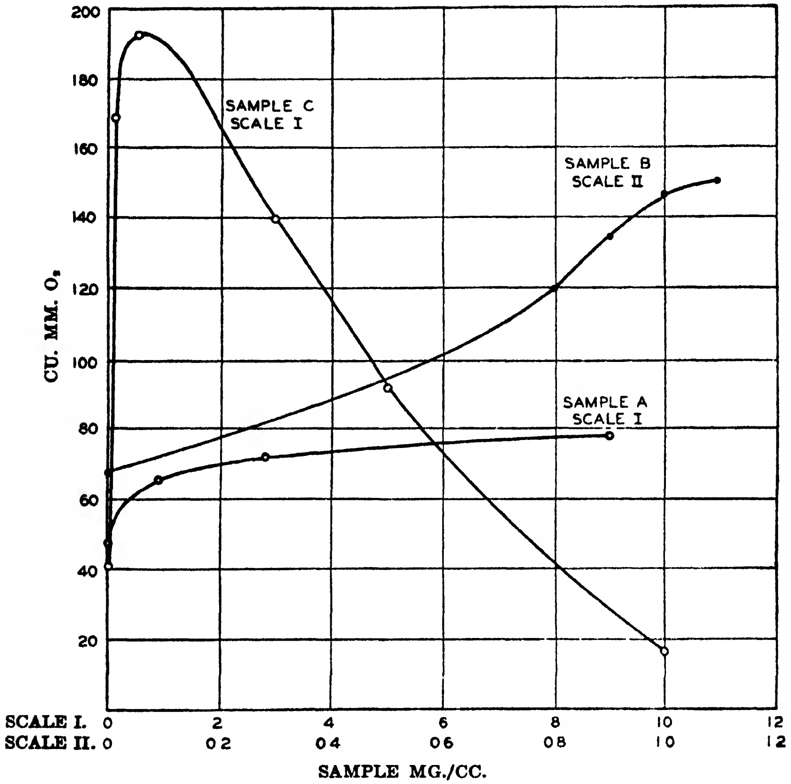
[1] Cook, E. S., and Kreke, C. W., THESE STUDIES, 2 (1938): 47.

[2] Cook, E. S., and Kreke, C. W., *Nature*, 142 (1938): 719.

[3] Cook, E. S., Kreke, C. W., and Nutini, L. G., THESE STUDIES, 2 (1938): 23.

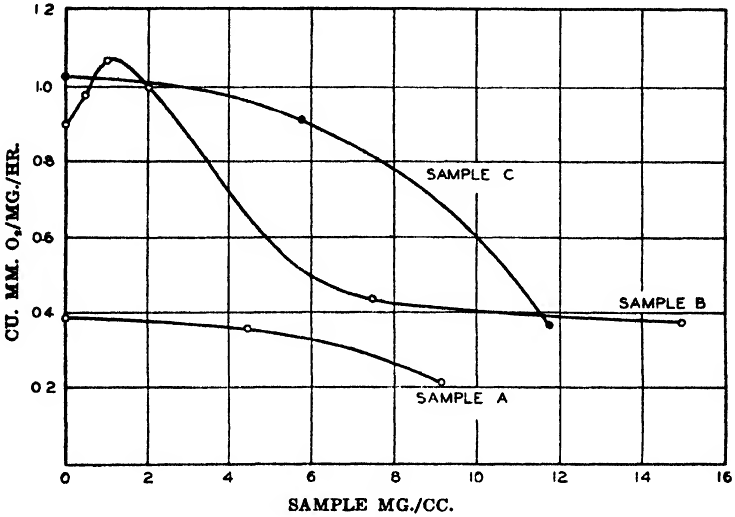
FIG. 1—*Respiratory Activity of Steam Distillate on Yeast*



All measurements on both yeast and skin were made at pH 7.3, the distillate being adjusted to this pH with sodium hydroxide. This treatment brought most of the distillate into solution.

The results of these determinations are shown in Fig. 1 and 2. It is seen that the successive methods of preparation gave products of increasing stimulatory activity for the respiration of yeast; in concentrations of 1 mg./cc., *Sample B* being about 3 times as active, and *C* nearly 9 times as active as *A*. (The percentage stimulations in this concentration are: *A* 39, *B* 116, *C* 341.) Very high concentrations, however, depress. All

FIG. 2—*Respiratory Activity of Steam Distillate on Rat Skin*



preparations depress the respiration of rat skin although in one set of experiments low concentrations appeared to show slight stimulatory activity (*Sample B*, Fig. 2). *Samples A* and *B* were slightly more potent than *C* in depressing skin respiration in concentrations of 10 mg./cc. (The percentage depressions in this concentration are: *A* 62 (extrapolated), *B* 57, *C* 43.) It is possible that the variation in potency is due not only to the different methods employed in preparation but also to the fact that different batches of yeast were employed for the several preparations. The authors have observed great variation in the potency of water-soluble respiratory-stimulating factors prepared from different samples of yeast.

It is of interest that preliminary experiments indicate that the steam distillate also depresses the respiration of transplants of a spindle cell carcinoma of mouse mammary gland*

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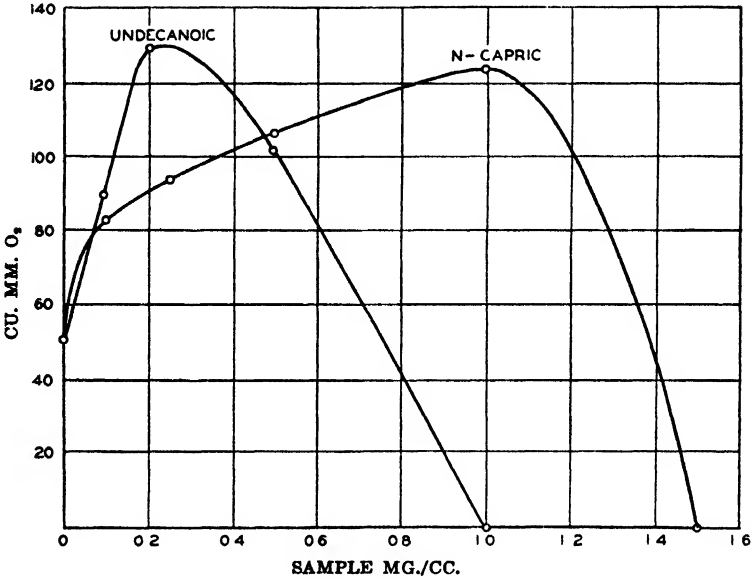
*Acknowledgment for this information is made to Sister M. Jordan Carroll, O.P., Rosary College Unit of Institutum Divi Thomae, River Forest, Illinois.

Fig. 3—*Respiratory Activity of n-Capric and Undecanoic Acids on Yeast*



(transplantable tumor No. 15091a, obtained from Jackson Memorial Laboratory). These observations on the depression of carcinoma transplants are based, however, on only five experiments.

Sample A was tested for its effect on anaerobic yeast fermentation according to the previously described technique.[4] It was found that in the concentrations of 5 to 10 mg./cc., which corresponded to those used for fermentation factors,[4] this sample was inhibitory to fermentation. Later work[5] has shown that, in these concentrations, capric acid is toxic to yeast (methylene blue staining). Therefore the action of the steam distillate here reported can undoubtedly be referred to

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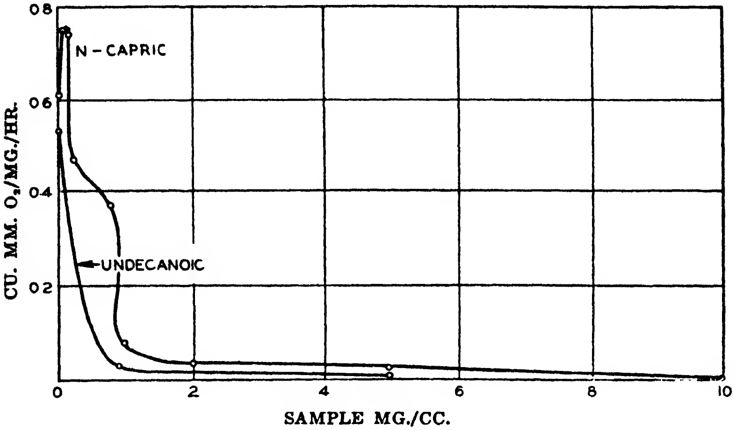
[4] Norris, R. J., and Kreke, C. W., These Studies, 1 (1937): 137.

[5] Cook, E. S., and Morgan, Sr. M. N., unpublished data.

FIG. 4—*Respiratory Activity of n-Capric and Undecanoic Acids on Rat Skin*



this toxic action and not to a specific inhibition of fermentation. Capric acid in concentrations under 1 mg./cc. appears to exert a slight stimulatory action on anaerobic fermentation.[5]

Fardon and Sullivan[6] studied *Samples A* and *B* (called A_3 and A_5, respectively, in their paper) and found them able to stimulate epithelial growth in tissue cultures when used in proper concentrations. High concentrations depressed growth. In connection with the observations recorded above it is of interest that *B* was more potent than *A* in the tissue culture experiments.

As mentioned earlier in this paper and elsewhere,[1, 2] the physical and chemical characteristics of the various steam distillates suggested that these were largely composed of saturated fatty acids. To investigate this possibility, two pure fatty acids, capric and undecanoic, were tested for their effect on yeast and skin respiration. At the pH of 7.3 used, these acids, of course, were present largely as the sodium salts. The results, shown in Fig. 3 and 4, demonstrate that these acids have actions qualitatively similar to those of the steam distillate samples. In other words, the fatty acids studied increase the respiration of yeast in non-toxic concentrations and depress the respiration of rat skin. A slight stimulation of skin respiration by pure capric acid in very low concentrations was found in all but one experiment. (Fig. 4.) This parallels the slight stimulation observed with a sample of distillate at low concentration. This effect seems to be outside of experimental error but without further data the authors feel they cannot be sure of its significance at this time, but it is of interest in connection with the experiments of Peters to be discussed later. In no case did the distillate or fatty acids consume oxygen themselves as was proved by tests in the absence of cells under the same experimental conditions. Further work[5] now in progress with different acids substantiates these conclusions.

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[6] Fardon, J. C., and Sullivan, W. A., THESE STUDIES, 2 (1938): 51.

DISCUSSION

It is not the purpose of this paper to elucidate the mechanism by which the steam distillate and fatty acids cause their respiratory effects: for this the data available at present are still insufficient. One of the authors, with the collaboration of Sr. M. Norbert Morgan, is now conducting experiments which they believe will throw light on the mode of action. However, it may not be amiss to call attention to several possible ways in which the fatty acids may act.

Several interpretations of the stimulatory action on yeast are possible. Among them are: (1) a direct chemical action on the respiratory enzyme systems, either catalytic (a coenzyme-like action), or a "short circuiting" of the normal enzyme chain; (2) a direct physical action on the cell due to the surface activity of the fatty acids and perhaps affecting such properties as permeability to glucose or other substrates; (3) an indirect "injuring" action leading to the production of respiratory factors by the cell; and (4) utilization of the fatty acids as substrates by the yeast.

No definite positive evidence exists for the first or direct catalytic action. The second mode of action is plausible in view of the known surface activity of fatty acids and undoubtedly is of importance. Adsorption to the cell wall could explain the observed effects as being the result of an alteration of permeability to oxidizable substrates. The permeability of the cell wall is a fundamental property which must be borne in mind. It will be recalled that Dixon and Holmes[7] have postulated a change in permeability for glucose as an explanation of the Pasteur effect, and Smythe[8] points out that certain aspects of the utilization of pyruvate by yeast are best explained on a similar basis.

In regard to the third possibility, it is well known that many physiologically toxic substances are stimulatory in low concentrations. Thus, in the authors' experience, 1,2,5,6-dibenzanthracene may exert a stimulatory or inhibitory effect on yeast respiration depending upon the concentration.[9] Similar results have been found by others for various respiratory-stimulating substances, such as nitrophenols.[10] It has been demonstrated that injury by physical means, as by ultraviolet irradiation, causes an increase in respiratory factors.[11]

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[7] Dixon, K. C., and Holmes, E. G., *Nature*, **135** (1935): 995; Dixon, K. C., *Biol. Rev. Cambridge Phil. Soc.*, **12** (1937): 431.

[8] Smythe, C. V., *J. Biol. Chem.*, **125** (1938): 635.

[9] Cook, E. S., Hart, Sr. M. J., and Joly, R. S., *Science*, **87** (1938): 331; *Am. J. Cancer*, **35** (1939): 543.

[10] Field, J., 2nd, Martin, A. W., and Field, S. M., *Proc. Soc. Exptl. Biol. Med.*, **34** (1936): 388; Krahl, M. E., and Clowes, G. H. A., *J. Biol. Chem.*, **111** (1935): 355; Clowes, G. H. A., and Krahl, M. E., *Proc. Soc. Exptl. Biol. Med.*, **34** (1936): 565; Bodine, J. H., and Boell, E. J., *ibid.*, **35** (1936-37): 504.

[11] Fardon, J. C., Carroll, Sr. M. J., and Ruddy, Sr. M. V., THESE STUDIES, **1** (1937): 17; Fardon, J. C., and Ruddy, Sr. M. V., *ibid.*, **1** (1937): 41; Fardon, J. C., Norris, R. J., Loofbourow, J. R., and Ruddy, Sr. M. V., *Nature*, **139** (1937): 589.

In this connection it is of interest that recent experiments[5] with pure fatty acids show that yeast cells subjected to inhibitory concentrations are nearly completely stained by methylene blue while cells treated with stimulatory concentrations do not stain. If injury is important in the stimulation of yeast respiration by fatty acids it is of insufficient intensity to kill many of the cells. This is in general agreement with the correlation of the production of growth factors by ultraviolet injury and killing.[12] As mentioned above, the toxic action may be due to the surface activity of the acids although, as will be pointed out later, the inhibiting effect on the respiration of some types of animal cells seems to be the result of direct toxic action on certain enzyme systems. Preliminary experiments[5] in an attempt to test this injury theory have shown that cell-free filtrates, obtained from yeast cells treated with capric acid, do show greater respiratory activity than the control filtrates from untreated cells. The magnitude of the effect, however, is quite insufficient to account for the respiratory stimulation observed with the direct introduction of fatty acids into the manometer flasks. It must be stressed that these experiments are preliminary and more potent cell-free filtrates may no doubt be obtained under proper conditions. However, from this evidence, together with some to be presented below, the authors do not believe that injury is the major source of respiratory stimulation of yeast by fatty acids.

The substrate theory must now be considered. As far as the authors are aware no account of the utilization of exogenous higher fatty acids by yeast has yet been published. The literature[13] does show that yeast converts acetate to fat, at the same time burning a part of it, but this does not appear to be true of formate, propionate or butyrate.[13a] Wieland[14] has reported that yeast oxidizes acetate to carbon dioxide and water, and also in part to succinic and citric acids under the proper conditions. Hence, it is quite possible that yeast is capable of oxidizing the higher fatty acids. It is well known that many animal tissues, such as liver, kidney and spleen, effect the oxidation of fatty acids *in vitro.*[15] In this connection it is of considerable interest that an optimum concentration of fatty acids exists for the respiration of liver slices, higher concentrations being inhibitory.[15] Pyruvic acid, which is converted to fat by yeast,[8] is also toxic to yeast in higher concentrations.[8, 16] Two types of experiments are now in progress[5] to investigate the ability of yeast to oxidize higher fatty acids: one, the measurement of the R.Q. of yeast with and without the addition of acid; and the other, the search for ketone bodies in the medium in which respiration takes place in the presence of acids. Preliminary evidence indicates the oxidation of the higher fatty acids by yeast. These experiments will be reported in detail later.

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[12] Loofbourow, J. R., Dwyer, Sr. C. M., and Morgan, Sr. M. N., THESE STUDIES, 2 (1938): 137; and unpublished data.

[13] (a) Smedley-Maclean, I., and Hoffert, D., *Biochem. J.*, 17 (1923): 720.

(b) Smedley-Maclean, I., and Hoffert, D., *ibid.*, 20 (1926): 343.

(c) Macleod, L. D., and Smedley-Maclean, I., *ibid.*, 32 (1938): 1571.

(d) Wieland, H., and Wille, F., *Ann.*, 515 (1935): 260.

For skin respiration the action of the distillate appears to be toxic, very possibly by the inhibition of a respiratory enzyme system or systems. Peters and Wakelin,* in a recent communication,[17] show that a number of sodium salts of fatty acids depress the oxidation of pyruvate by avitaminous brain *brei* either in the presence or absence of vitamin B_1. In no concentration was stimulation observed. They have shown that the action is due to a direct inhibitory effect on the pyruvate oxidase system, apparently by displacement of one of the members of the system. A much slighter effect was demonstrated on the succinoxidase system. This work, of course, suggests that in the authors' experiments the inhibitory action of the steam distillate on skin respiration is due to direct action on the respiratory enzyme systems, although the physical actions discussed above are undoubtedly of importance for the penetration of the acids into the cell. Ciaranfi[18] has shown that a variety of tumor tissues do not oxidize fatty acids *in vitro* and some acids are inhibitory to tumor respiration. This checks with Carroll's observation of the inhibition of tumor respiration by the steam distillate.[19]

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[14] Wieland, H., and Sonderhoff, R., *Ann.*, 499 (1932): 213.

[15] Quastel, J. H., and Wheatley, A. H. M., *Biochem. J.*, 27 (1933): 1753; 28 (1934): 1014; Jowett, M., and Quastel, J. H., *ibid.*, 29 (1935): 2143, 2159, 2181.

[16] Haehn, H., and Glaubitz, M., *Z. physiol. Chem.*, 168 (1927): 233.

*The authors are greatly indebted to Professor Peters for a proof copy of his paper.

[17] Peters, R. A., and Wakelin, R. W., *Biochem. J.*, 32 (1938): 2290.

Experiments are now in progress with pure fatty acids in an attempt to answer some of the questions discussed in this paper.

SUMMARY

Steam distillation of an aqueous-alcoholic extract of yeast yields a small amount of a water-insoluble substance which appears to consist largely of a mixture of saturated fatty acids. This distillate in non-toxic concentrations, as determined by methylene blue staining, markedly increases the oxygen consumption of yeast suspensions. It inhibits the respiration of rat skin but leads to increased epithelial growth in tissue culture in proper concentrations.

Capric and undecanoic acids exhibit a qualitatively similar behavior on yeast and skin respiration.

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[18] Ciaranfi, E., *Am. J. Cancer*, 32 (1938): 561.

[19] Page 217.

LITERATURE CITED

Bodine, J. H., and Boell, E. J., *Proc. Soc. Exptl. Biol. Med.*, **35** (1936-37): 504.

Ciaranfi, E., *Am. J. Cancer*, **32** (1938): 561.

Clowes, G. H. A., and Krahl, M. E., *Proc. Soc. Exptl. Biol. Med.*, **34** (1936): 565.

Cook, E. S., and Kreke, C. W., These Studies, **2** (1938): 47; *Nature*, **142** (1938): 719.

Cook, E. S., Kreke, C. W., and Nutini, L. G., These Studies, **2** (1938): 23.

Cook, E. S., Hart, Sr. M. J., and Joly, R. S., *Science*, **87** (1938): 331; *Am. J. Cancer*, **35** (1939): 543.

Dixon, K. C., *Biol. Rev. Cambridge Phil. Soc.*, **12** (1937): 431.

Dixon, K. C., and Holmes, E. G., *Nature*, **135** (1935): 995.

Fardon, J. C., Carroll, Sr. M. J., and Ruddy, Sr. M. V., These Studies, **1** (1937): 17.

Fardon, J. C., Norris, R. J., Loofbourow, J. R., and Ruddy, Sr. M. V., *Nature*, **139** (1937): 589.

Fardon, J. C., and Ruddy, Sr. M. V., These Studies, **1** (1937): 41.

Fardon, J. C., and Sullivan, W. A., These Studies, **2** (1938): 51.

Field, J., 2nd, Martin, A. W., and Field, S.M., *Proc. Soc. Exptl. Biol. Med.*, **34** (1936): 388.

Haehn, H., and Glaubitz, M., *Z. physiol. Chem.*, **168** (1927): 233.

Jowett, M., and Quastel, J. H., *Biochem. J.*, **29** (1935): 2143, 2159, 2181.

Krahl, M. E., and Clowes, G. H. A., *J. Biol. Chem.*, **111** (1935): 355.

Loofbourow, J. R., Dwyer, Sr. C. M., and Morgan, Sr. M. N., These Studies, **2** (1938): 137.

Macleod, L. D., and Smedley-Maclean, I., *Biochem. J.*, **32** (1938): 1571.

Norris, R. J., and Kreke, C. W., These Studies, **1** (1937): 137.

Peters, R. A., and Wakelin, R. W., *Biochem. J.*, **32** (1938): 2290.

Quastel, J. H., and Wheatley, A. H. M., *Biochem. J.* ,**27** (1933): 1753; **28** (1934): 1014.

Smedley-Maclean, I., and Hoffert, D., *Biochem. J.*, **17** (1923): 720; **20** (1926): 343.

Smythe, C. V., *J. Biol. Chem.*, **125** (1938): 635.

Wieland, H., and Sonderhoff, R., *Ann.*, **499** (1932): 213.

Wieland, H., and Wille, F., *Ann.*, **515** (1935): 260.

Original Manuscript submitted
December 12, 1938.

CHARACTERISTICS OF PHOTOGRAPHIC MATERIALS IN THE ULTRAVIOLET

PART I—VARIATION OF CONTRAST WITH WAVELENGTH FOR EASTMAN PAR SPEED PORTRAIT FILM

BY JOHN R. LOOFBOUROW

IN the application of photographic methods to heterochromatic spectrophotometry in the ultraviolet region, it is important to know the characteristics of photographic emulsions in the ultraviolet. Little material has, however, been published on this subject. A study of the characteristics of several emulsions in the ultraviolet was published by Harrison[1] some time ago, and since then Harrison and Leighton[2] have presented additional data in their papers concerning the use of fluorescent materials to increase the sensitivity of plates to the extreme ultraviolet.

In order to accumulate more complete information, the author has undertaken the study of a number of photographic emulsions from the standpoint of the variation of sensitivity, contrast and reciprocity-law failure with wavelength in the ultraviolet region. This work was started when the author was at the Basic Science Research Laboratory of the University of Cincinnati and has been continued at the present institution. It is planned to publish the results of these studies in a series of papers, of which this is the first.

The present paper is concerned with the variation of contrast with wavelength of Eastman Par Speed Portrait Film, throughout the range 2300-3600 Å. The films were exposed to the radiation from a tungsten spark source in a small Gaertner quartz spectrograph, using a slit width of approximately 0.03 mm. In the data reported here, the exposure was controlled by varying the time at constant intensity. Subsequent reports will show the departure from the reciprocity law for this emulsion throughout the range of wavelengths employed. The characteristic curves obtained by variation of time are useful both in choosing suitable exposure times in spectrophotometry by such methods as the Hilger Spekker photometer, and in applications to spectrophotometric methods in which density is controlled by time of exposure.[3] A tungsten spark source was employed because of its widespread use in ultraviolet absorption spectrophotometry and because of its richness in lines in the ultraviolet region.

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[1] Harrison, G. R., *J. Optical Soc. Am.*, **11** (1925): 341.

[2] Harrison, G. R., and Leighton, P. A., *J. Optical Soc. Am.*, **20** (1931): 313; *Phys. Rev.*, **38** (1931): 899.

The condensed tungsten spark, using one-eighth inch electrodes, was operated at 30,000 volts by a 500-watt transformer. The dispersion of the spectrograph was such as to give a spectrum length of 2.6 cm. between 4000 Å and 2000 Å. It employed a 60° Cornu prism of 2 x 3 cm. face, and collimator and camera lenses with relative aperture approximately *f*.12. These data serve as a basis for computing exposures with other instruments to obtain values for match points in the straight line portions of the exposure curves in the match-point method of photographic spectrophotometry.

While the tungsten spark is an intermittent source, the primary flashes are of high frequency. It has been shown by Webb[4] that at high frequencies the intermittency effect is negligible. Probably of greater importance from the standpoint of the intermittency effect is the operation of the source from a 60-cycle alternating current supply. Nevertheless, it

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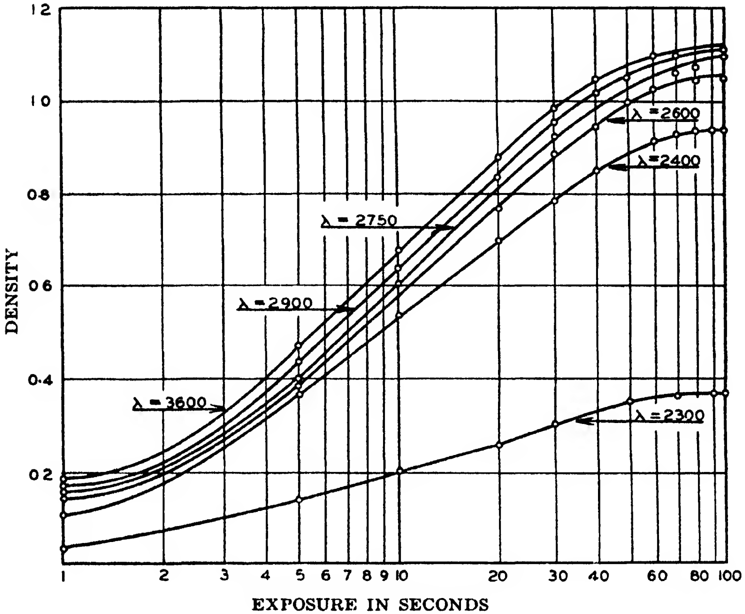
[3] Loofbourow, J. R., *Bull. Basic Sci. Research,* 5 (1933): 46.

[4] Webb, J. H., *J. Optical Soc. Am.,* 23 (1933): 157; 26 (1936): 347.

FIG. 1—*Characteristic Curves of Eastman Par Speed Portrait Film in the Ultraviolet*



is doubtful if it would be advisable to employ a rectified and filtered constant-potential high-voltage supply, because in ordinary spectrophotometric applications such equipment is not used. It is the author's aim to collect practical data which will be of service to those working in the field of ultraviolet absorption spectra.

The films were developed in Rodinal, 1:50, for 6 minutes at 20° C. The film densities were measured by a projection photoelectric photodensitometer employing a collimated light beam. The densities obtained with collimated light are not the same as those obtained with pot opal or integrating sphere densitometers, as has been shown by Koerner and Tuttle.[5]

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[5] Koerner, A. W., and Tuttle, C., *J. Optical Soc. Am.*, 27 (1937): 241.

TABLE I

Values Added to Density Values in Plotting Characteristic Curves of Fig. 1

Wavelength in Å	Value Added to Densities
3600	+0.097
2900	+0.08
2750	+0.092
2600	+0.053
2400	−0.04
2300	0

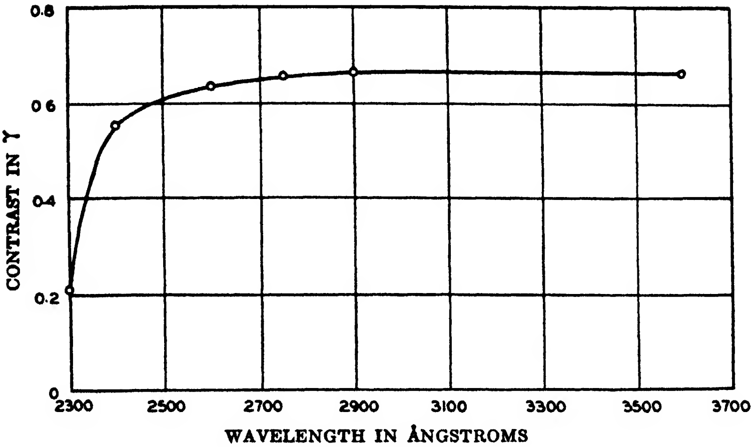
Nevertheless, the departure from pot opal readings is not sufficiently great to be of importance in the present study.

Characteristic curves of the emulsion in question are shown in Fig. 1. In order to prevent overlapping of these curves, the ordinates have been changed slightly by adding or subtracting the values shown in Table I from the density readings in plotting the curves.

The intensities used varied throughout the spectrum both due to the spectral energy characteristics of the source and the dispersion characteristics of the instrument. No correction to constant intensity was used in expressing the results because it was desired that they represent conditions actually encountered in spectrophotometric practice with quartz spectrographs.

The curves of Fig. 1 show (after allowance for the changes in ordinates used in plotting) that the densities obtained with a given time of exposure throughout the range 3600 Å to 2400 Å are closely similar, under the conditions employed in the experiments. The contrast begins to decrease below 2600 Å, in agreement with the results found by Harrison[1, 2] for various emulsions. At 2400 Å the decrease in contrast is quite marked.

FIG. 2—*Relation of Contrast to Wavelength for Eastman Par Speed Portrait Film in the Ultraviolet*



The relation of contrast to wavelength is shown in Fig 2, where γ has been plotted against wavelength for the conditions of exposure and development used.

The decrease in contrast below 2400 Å is such as to make the use of this emulsion for photographic match-point spectrophotometry in the short-wave region inadvisable unless fluorescent materials[2] are used in combinations with the emulsion to improve its response in this region.

The uniformity of contrast and uniformity of density obtained for equal times of exposure with a quartz spectrograph and tungsten spark source in the wavelength range 3600 Å to 2500 Å show that the emulsion is well suited to match-point spectrophotometry in this region.

Original Manuscript submitted
December 20, 1938.

GROWTH-PROMOTING SUBSTANCES LIBERATED BY TRAUMATIZED TISSUES IN VITRO

By JOHN C. FARDON, REV. WILLIAM A. SULLIVAN, O.P., AND
SISTER M. BASILIA ANDRUS, O.P.

Since the relation of cell injury and degeneration to the liberation of growth-promoting substances has been discussed in a previous paper,[1] a review of the authors' work and that of others will not be attempted in its entirety. The purpose of this communication is merely to substantiate the authors' contention formulated in their last paper. The technical difficulties of injuring tissues by cutting and distinguishing in the subsequent cultures the exact areas so traumatized have been successfully circumvented.

Observations made by Fischer[2a] confirm the idea that injured cells liberate growth stimulators to a greater extent than do completely destroyed cells. He observed, in his preparations of embryonic tissue juice, that when the embryos were cut into bits with a pair of scissors greater activating power was displayed than when the embryos were ground with sand or "kieselgur" in a mortar. It is obvious from this that if destroyed cells produced a greater amount of growth-promoting substance, then grinding the tissue with sand should have given a more potent fraction, for greater destruction of cells could be effected in this manner. The authors repeated these experiments and obtained the same results as Fischer. Experiments by Fischer[2b] on the healing of wounds *in vitro* show that after successive traumatization the culture grows definitely faster than the undamaged control cultures. He also observed that the speed with which wound healing takes place in such cultures can be measured and is inversely proportional to the age of the culture.

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[1] Fardon, J. C., and Sullivan, W. A., These Studies, 2 (1938): 39.

[2] (a) Fischer, A., Tissue Culture, Levin and Munksgaards, Copenhagen, 1917 p. 84.

(b) Fischer, A., *Virchow's Arch. path. Anat.*, 279 (1930): 94.

Using suspensions of yeast cells, Loofbourow[8] found that the filtrate from a suspension irradiated by low-intensity ultraviolet produced more abundant growth of yeast cells than did the filtrate from a suspension subjected to a high intensity. These and similar experiments have demonstrated that injured cells produce greater amounts of the proliferation-stimulating factors than do killed cells.

To demonstrate with any degree of directness *in vitro* that injured tissues liberate growth-promoting substances entails several technical difficulties. To so cut and orient a fragment of tissue in plasma and later be able to recognize the cut portion, often introduces much doubt. Again, having been fortunate enough to keep an accurate record of the injured portion, it frequently happens that the resultant growth and migration of cells fashion a pattern which to all appearances gives evidence of uniform homogeneous growth from all portions of the tissue. Another important phenomenon that must be given due consideration, when measuring growth as a result of injury, is cell division and migration from certain definite loci of the explanted tissue fragment. One may assume, and justifiably, that all parts of a developing organism do not differentiate and proliferate at the same time and at the same rate. During the developmental period some cell centers can always be found which are passive and other centers which are actively growing. These independent loci of active proliferation have been found along various portions of the

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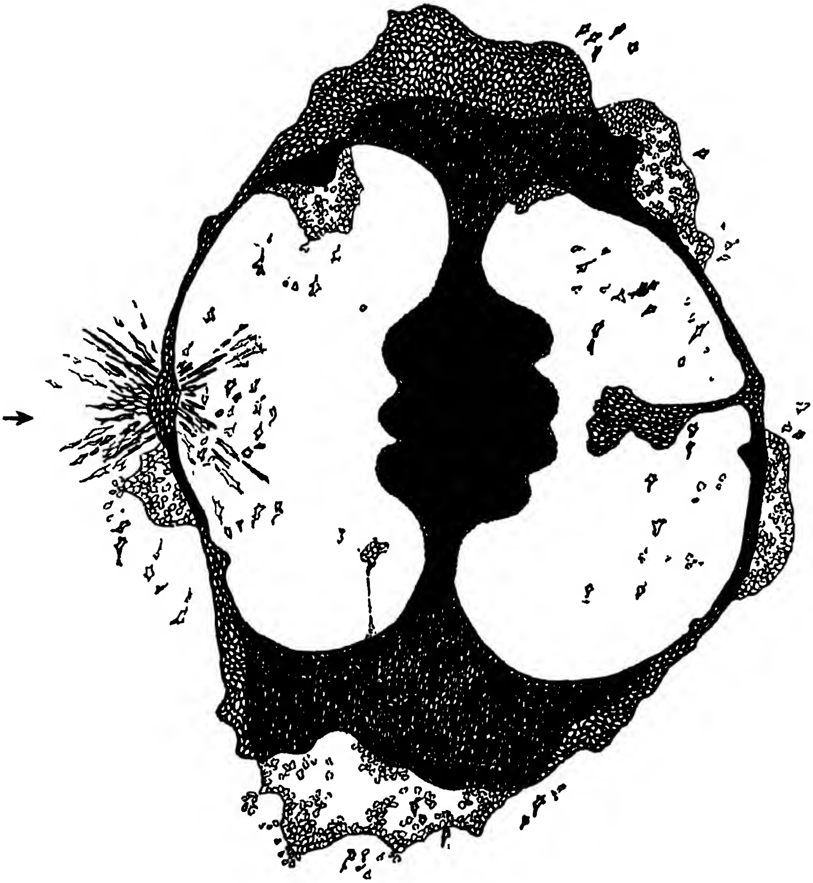
[8] Loofbourow, J. R., Dwyer, Sr. C. M., and Morgan, Sr. M. N., THESE STUDIES, 2 (1938): 137.

Fig. 1—*Diagram Showing Nature of Outgrowth*



embryonic intestine (Fig. 12) and even in the outgrowth of the original tissue explant (See arrow in Fig. 1; also Fig. 10 and 11).

EXPERIMENTAL

Some 200 culture slides were prepared for the experiment, using as the culture medium only Drew's solution and chicken plasma in equal proportions. Embryos were removed from eggs under aseptic conditions and the intestines removed and cleansed in Drew's solution. The ages of the embryos varied from 14 to 18 days. The intestine was carefully cut in transverse sections not exceeding 2 mm. in length. In transferring the sections to the cover slips, special precautions were taken not to injure any portion of the muscular coat of the intestinal wall. Each fragment of intestine was so placed on the cover slip that the two cut ends were clearly visible. The culture medium was not replenished and the slides were discarded when signs of degeneration became evident. Observations were usually taken every 12 hours, and photomicrographs taken after 65 and 113 hours of incubation. In the majority of cases active peristalsis continued until shortly before degeneration of the culture. In several cases where the villi of the intestines were exposed, active motion of the cilia could be observed.

As shown in the photomicrographs, active growth in every instance takes place at the cut ends (indicated by lines) of the intestine. This growth appears to be composed of connective and epithelial tissue with a few scattered muscle cells. Some migration of fibrocytes has taken place throughout the immediate plasma clot. It will be noted that before the occurrence of liquefaction of the plasma clot the outgrowth from the original piece of intestine is fan-shaped. With the onset of liquefaction, however, two complete rings or loops of growth appear which connect both ends of the section of intestine. (See Fig. 6, 8, 9. Fig. 13 shows the cellular composition of a section of the ring). This phenomenon is usually referred to as the "ring of liquefaction" or "signet ring." Lambert and Hanes[4] suggest that such a ring might be explained by the action of proteolytic ferment on the fibrin of the plasma, allowing the contracting plasma to retract from the embedded

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[4] **Lambert, R. A., and Hanes, F. M., *J. Exptl. Med.*, 13 (1911): 495.**

PLATES I

Cultures of Embryonic Chick Intestine

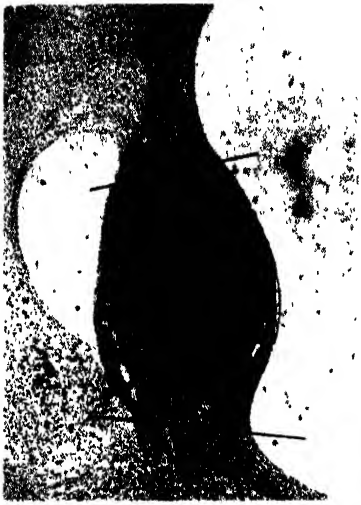


Fig 2

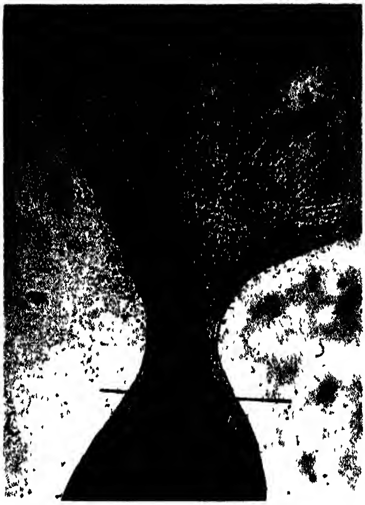


Fig 3

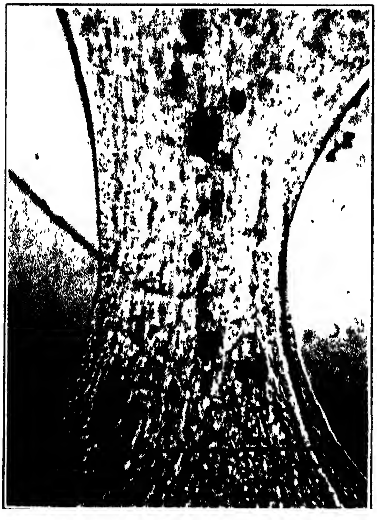


Fig. 4

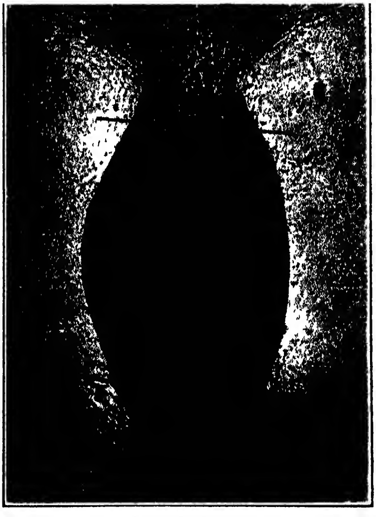
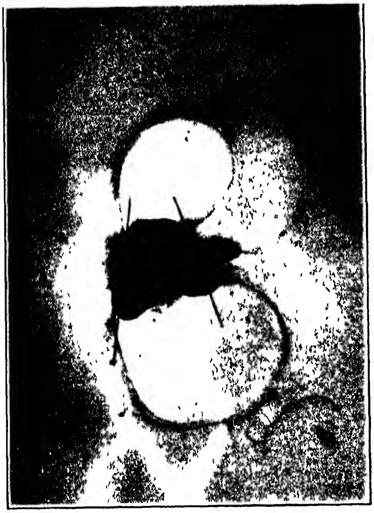


Fig. 5

PLATES II

Cultures of Embryonic Chick Intestine



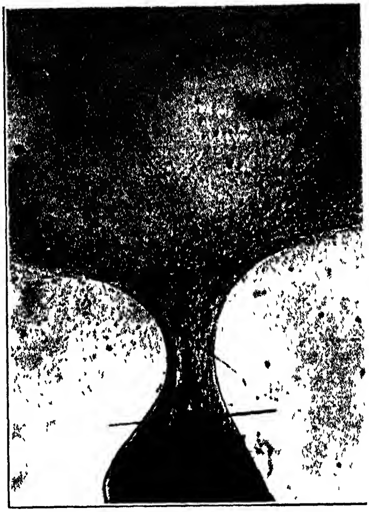


Fig 7

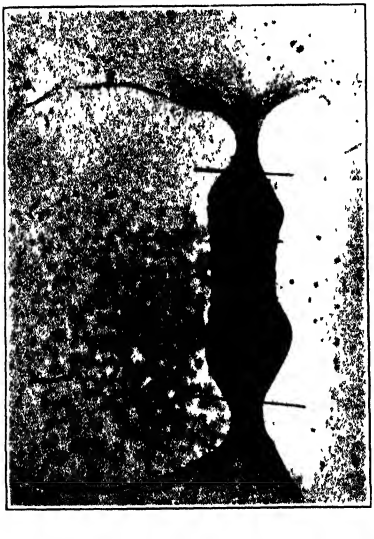
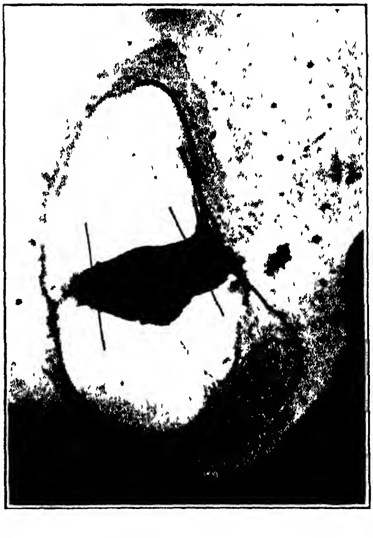


Fig 8



PLATES III

Cultures of Embryonic Chick Intestine

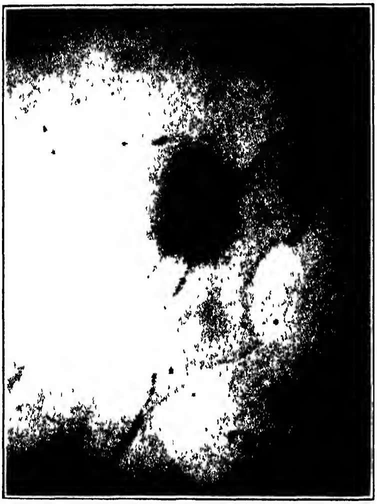


Fig 10

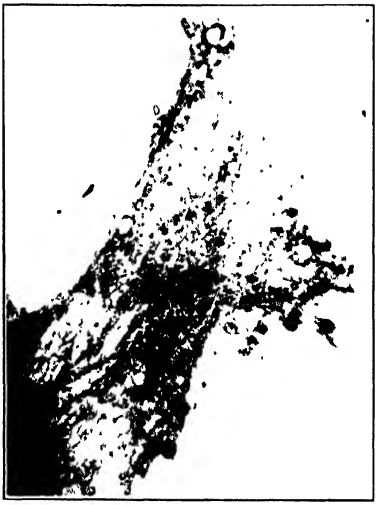


Fig 11

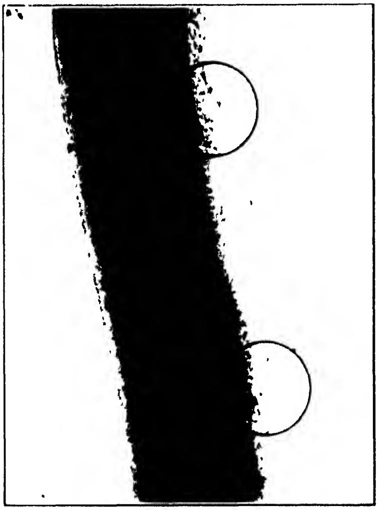


Fig 12

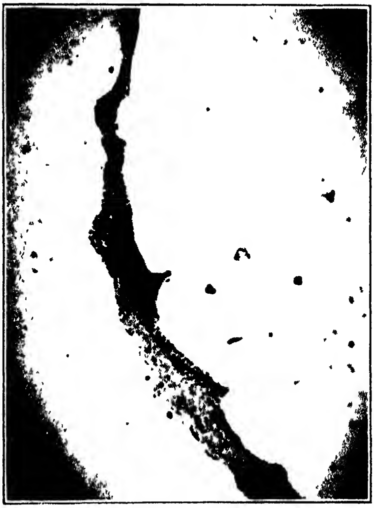


Fig 13

tissue. Within the ring the plasma is definitely liquid and more transparent than the clot. This ring of liquefaction is most frequently observed in cultures of tumor tissue and occasionally in adult tissue. Whether, in the case of embryonic intestine cultures, this ring is mainly a result of liquefaction, or whether some affinity exists between the outgrowing cells of both ends of the segment, is not yet known. However, the outgrowth is not merely a sheet of connected cells, but in nearly every case is most decidedly a split tubular sort of growth which could hardly be attributed to the liquefaction phenomenon. The nature of this peculiar growth might easily be compared to a piece of rubber tubing, which having been slit longitudinally for some distance at either end, had these ends looped back and connected. This picture is most readily observed if the microscope is focused alternately upon the two layers of pavement epithelium. Since a photomicrograph of this type of growth could not be taken because of the depth of focus required for good definition, a drawing is shown in Fig. 1 to illustrate this as accurately as possible. One can easily conclude from this picture that the effort here is one of differentiation and continued adherence to (in so far as conditions permit) organized growth. The continued differentiation is nicely demonstrated in Fig. 4 (left of the line) which shows a portion of organized new growth.

CONCLUSION

In tissue cultures *in vitro* growth proceeds most actively from that portion of tissue which has been subjected to injury by cutting.

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April 12, 1939.

SOLUBILITY BEHAVIOR OF RESPIRATORY FACTORS FROM YEAST

By ELTON S. COOK AND ELSIE M. WALTER

THE purpose of the present work was to study the efficiency of extraction processes previously used in the preparation of respiratory factors from yeast and to investigate the effects of some common solvents on relatively crude respiratory concentrates with a view to eliminating inactive materials from the concentrates.

I.

A previously described[1] method of extracting respiratory-stimulating factors consisted of treating Fleischmann's bakers' yeast with 95 per cent ethyl alcohol and then with 50 per cent ethyl alcohol at 60°-70° C. for 4 hours in each case. For every 7 pounds of yeast 2.5 liters of alcohol were used. The extracts were then combined and concentrated by vacuum distillation at 26-29 inches and at a temperature below 60° C. The concentrated material was the crude respiratory-stimulating factor.

In this study the relative efficiency of the two extractions was determined by concentrating separately the 95 per cent and the 50 per cent alcohol extracts and by testing them separately and combined in the proportion which would exist if the two extracts had been combined before concentration. It is to be recalled that the cake yeast itself contains about 80 per cent water so that the final alcoholic concentration of the first extract is considerably lower than 95 per cent.

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[1] Cook, E. S., Kreke, C. W., and Nutini, L. G., THESE STUDIES, 2 (1938): 23.

TABLE I

Comparison of Alcoholic Extractions

Batch	Weight of Yeast Extracted in g.	Extract	Yield in g.	Yeast Activity		Skin Activity	
				Per Cent Stimulation	Concentration in mg./cc.	Per Cent Stimulation	Concentration in mg./cc.
3	4540 (10 lb.)	95% Ethyl Alcohol	168	16	1.0	43 48	10.0 13.3
		50% Ethyl Alcohol	47	27	1.0	17 11	6.2 10.0
		Combined	215	16	1.0	19	6.5
4	9530 (21 lb.)	95% Ethyl Alcohol	349	16	1.0	33 33	6.8 13.6
		50% Ethyl Alcohol	123	14	1.0	14 14	6.2 12.4
		Combined	472	—	—	23 23	6.5 13.0
5	9530 (21 lb.)	95% Ethyl Alcohol	431	59	1.0	30 30	6.7
		50% Ethyl Alcohol	109	57	1.0	32	13.4 12.7
		Combined	540	55	1.0	35 35	6.7 13.3

The individual extracts and the combined extracts were tested for their effects on the respiration of rat skin and yeast. The skin and yeast experiments were performed in the usual manner which has been described by Cook, Kreke and Nutini.[1] Seven manometer flasks were generally used. The first served as a temperature and barometric control, the second and third as skin or yeast controls, as the case may be, the fourth and fifth contained the sample in high concentration (12-15 mg./cc. for skin, 1 mg./cc. for yeast), and the sixth and seventh contained the sample in low concentration (5-8 mg./cc. for skin, 0.5 mg./cc. for yeast). Table I gives the results on three batches of material.

The table shows that the 95 per cent alcohol extract contains from three to four times more material by weight than the 50 per cent alcohol extract. In general, the material in the two extracts is equally active on yeast respiration while there is some times a drop in skin activity in the second extract. A material saving in time could be accomplished by omitting the second extraction. Hence, the amount of alcohol was increased from 2.5 to 3 liters per 3.2 Kg. (7 lb.) of yeast and the second extraction eliminated. This gave yields equal to those of the combined extracts in the earlier experiments. The combined yields in the above experiments constitute from 4.7 to 5.7 per cent of the original wet yeast, which is slightly higher than that obtained in the earlier experiments.[1] The table further illustrates the variability in respiration activity of different batches of yeast. It also shows that with a given sample of respiratory factor there is frequently a concentration limit beyond which it is useless to go when attempting to stimulate skin respiration. This has been found to be true with the respiratory factor partially purified by adsorption techniques.[2]

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[2] Cook, E. S., and Walter, E. M., This Issue, p. 189.

II.

The second part of the study deals with the solubility of the respiratory factors in various concentrations of ethyl alcohol and in ether, acetone and methyl alcohol.

Preliminary experiments dealt only with ethyl alcohol. They showed that the addition of ethyl alcohol to aqueous solutions of the crude respiratory factors caused the formation of a precipitate when alcohol concentrations above 40 per cent were used. The quantity of precipitate increased with rising alcohol concentration so that only about 10 per cent of the material was soluble at alcohol concentrations of 95 per cent. In a number of cases samples of the insoluble material were filtered off immediately after precipitation and respiration assays made. In general it appeared that, while part of the alcohol-insoluble material was inactive, high concentrations of alcohol also caused the precipitation of the factors stimulating yeast and skin respiration. However, the results of individual experiments were not quantitatively consistent, possibly due to variation in relative amounts of active and inactive materials. It was found later that this was also partly due to failure in allowing sufficient time for equilibrium to be reached during the precipitation.

A second set of experiments was carried out in which, instead of adding ethyl alcohol to aqueous solutions of the factors, solid respiratory factor concentrates were treated with various solvents for varying periods of time. Consistent results were obtained by this procedure. The respiratory factor used in these experiments had been partially purified by treatment with Norit charcoal in a water solution at 60° C. as described in a previous study.[2] Twenty-five cubic centimeters of the purified factor in a concentration of 102 mg./cc. were placed in each of seven beakers and taken to dryness in the oven at 50° C. To each beaker, after weighing, were added

TABLE II

Solubilities of Rat Skin Respiratory Factor

Solvent	Sample	Time in Hours	Weight in Per Cent	Relative Respiratory Activities of the Two Fractions Compared With Each Other	Relative Respiratory Activities Compared With Original Material
Diethyl Ether	Soluble	3	0*	0	0
	Insoluble		100	100	100
Acetone	Soluble	24	0*	0	0
	Insoluble		100	100	100
Methyl Alcohol	Soluble	72	61	78	200
	Insoluble		39	22	50
100% Ethyl Alcohol	Soluble	24	0.5	0	0
	Insoluble		99.5	100	100
90% Ethyl Alcohol	Soluble	3	10	44	83
	Insoluble		90	56	100
80% Ethyl Alcohol	Soluble	3	37	55	95
	Insoluble		63	45	80
70% Ethyl Alcohol	Soluble	3	77	70	120
	Insoluble		23	30	50

*Soluble material amounted to 0.2—0.6 mg. out of a total of 2.6 g.

75 cc. of the solvent to be studied and the beakers were allowed to stand for the periods of time shown in Table II. The insoluble material was then filtered off and washed with an additional 25 cc. of the solvent and these washings were added to the filtrate. Table II shows in per cent the division of the weights and skin respiratory activities between the dissolved and undissolved portions of each of the seven solvents. In Column 5 the respiratory activities per unit weight are compared for each pair of fractions, the sum of the activities of the soluble and insoluble portions being taken as 100. In Column 6 the relative activities of the fractions per unit weight are compared with the activity of the starting material, which is taken as 100.

It is seen that the respiratory factor and associated inactive material are insoluble in absolute ether and acetone and are practically insoluble in absolute ethyl alcohol. The solubility of both materials increases as the concentration of ethyl alcohol decreases. The active material appears to be slightly less soluble in 90 per cent ethyl alcohol than is the inactive. At concentrations of 80 per cent or below, the soluble portion is more active biologically. In all of the effective ethyl alcohol concentrations, except 70 per cent, the activity per unit weight is distributed approximately equally between the soluble and insoluble portions so that no purification from inactive contaminants is secured. Although the 70 per cent ethyl alcohol soluble portion is about 2.5 times as active as the insoluble portion, it actually represents only a 20 per cent increase in activity over the original material.

Absolute methyl alcohol is the most promising of the solvents. The 39 per cent of insoluble material is only half as active as the untreated respiratory factor and hence contains a fair proportion of inert substances. The remaining 61 per cent is twice as active per unit weight as the original sample and thus represents a definite increase in purity. Further study should be made of the use of methyl alcohol in the purification of respiratory factors.

SUMMARY

The relative efficiencies of the successive 95 per cent and 50 per cent ethyl alcohol extractions of yeast in the preparation of respiratory factors by a previously described method[1] have been compared. The first extraction is three to four times as effective as the second.

The respiratory factor is insoluble in absolute ether and acetone and substantially insoluble in absolute ethyl alcohol. Varying amounts of insoluble material are obtained with different concentrations of ethyl alcohol but satisfactory separation of active and inactive constituents is not accomplished. Absolute methyl alcohol is the most promising solvent tried, the active materials being relatively more soluble in it than the inactive ones.

Original Manuscript submitted
June 24, 1939.

THE EFFECTS OF TISSUE EXTRACTS ON THE RESPIRATION AND GLYCOLYSIS OF SOME NORMAL AND TUMOR TISSUES*

By SISTER M. PETRONELLA SCHROEDER, C.PP.S. AND
ELTON S. COOK

THE classical work of Warburg on the metabolism of tumor tissues may be summarized in the following words of Dean Burk:[1] "The difference between ordered and unordered growth lies in the relation between respiration and glycolysis, in that in unordered tumor growth respiration does not succeed in preventing glycolysis. *A tumor is a growing glycolyzing tissue with a deficient respiration.*" In other words, from a metabolic point of view, tumor tissue appears to be characterized by a defective Pasteur reaction, although Warburg made a provisional classification of tissues essentially independent of the Pasteur effect. Warburg has gone so far as to state:[2] "Interference with the respiration in growing cells is, from the standpoint of the physiology of metabolism, the cause of tumours." This causal relationship does not seem to have been adequately demonstrated, although certain facts may be taken as supporting this theory. Among these are the reports that the tissues of susceptible mice show lowered respiration,[3] and that lowered respiration is found prior to tumor development in the skin of mice irradiated with X-rays or injected with methylcholanthrene.[4]

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*Research conducted under the Sir Charles F. Williams Fellowship.

[1] Burk, D., in Some Fundamental Aspects of the Cancer Problem, Science Press, New York, 1937, p. 121.

[2] Warburg, O., (Trans. by F. Dickens), The Metabolism of Tumours, Constable, London, 1930.

[3] Büngeler, W., *Frankfurt. Z. Path.*, 39 (1930): 314; Davis, J. E., *Can. Med. Assoc. J.*, 36 (1937): 27.

In other respects, also, the original concepts of Warburg have been extended and modified. The fact remains, however, that from the metabolic standpoint, tumor tissues are most generally differentiated from normal tissues by possession of a defective ratio of respiration to glycolysis. The direct relationship of these two properties to the rate of growth of tumors has been questioned.[5] Work in our laboratories on the production of proliferation-promoting factors may throw light on this aspect of the problem and will be discussed in other papers. The metabolic abnormalities of tumor tissue require further study to determine the actual part which they play in the cancer problem. The isolation in our laboratories of respiratory-stimulating factors from a variety of cells provides an approach to this study. The object of the present paper is to investigate the effects of a number of these respiratory factors on the respiration and glycolysis of normal and tumor tissues.

The importance of the Pasteur effect in relating respiration and glycolysis in normal and tumor tissues is well recognized. Investigation of the mechanism of the Pasteur reaction[1, 6] has been partly concerned with the possible independence of the respiratory and glycolytic processes. Although it was originally supposed that the glycolytic process must precede respiration, evidence is accumulating that the two processes may be independent. Evidence for this is based mainly upon the fact that it is possible completely to inhibit anaerobic glycolysis with substances such as iodoacetate and yet permit the respiration of carbohydrate to proceed.[7] The present work shows that the addition of certain respiratory factors may actually increase respiration and at the same time partially inhibit anaerobic as well as aerobic glycolysis. These results tend to support the belief that glycolysis need not precede respiration although the investigations must be extended before complete confirmation of this belief is possible.

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[4] Fardon, J. C., and Sullivan, W. A., *Nature*, 143 (1939): 287; and Fardon, J. C., Sullivan, W. A., Brotzge, G. C., Loeffler, Sr. M. K., and Andrus, Sr. M. B., THIS ISSUE, p. 203.

[5] Murphy, J. B., and Hawkins, J. A., *J. Gen. Physiol.*, 8 (1925): 115; Bancroft, G., Beck, L. V., and Russell, M. A., *Biochem. J.*, 29 (1935): 2416; Crabtree, H. G., *ibid.* 23 (1929): 536; Boyland, E., and Boyland, M. E., *ibid.*, 33 (1939): 618.

[6] Dixon, K. C., *Biol. Rev.*, 12 (1937): 431.

Büngeler[8] in 1932 studied the effects of extracts prepared from spleen and a few other tissues on the respiration and glycolysis of several mouse tumors. He found that these extracts vary in activity. Mouse spleen extract had by far the greatest activity in stimulating respiration and depressing glycolysis. His results qualitatively resemble our own.

Carroll[9] found that "crude" yeast extracts were ineffective in stimulating the respiration of transplants of a spindle-cell carcinoma of the mouse mammary gland and confirmed some of Büngeler's findings. Later unpublished experiments by Carroll have shown that purified yeast extracts of the type used in the work described in the present paper are able to increase the oxygen uptake of the same spindle-cell carcinoma.

EXPERIMENTAL

The respiratory-stimulating factors used in this study were obtained from both yeast and animal tissues. Two samples of the factor from yeast were prepared in our laboratories: the "crude" sample was prepared by extraction with alcohol in the manner described by Cook, Kreke and Nutini[10] and was sterilized by Berkefeld filtration; the other sample was obtained in the same way but was further purified by a twofold treatment with charcoal at 60° C., as described by Cook and Walter.[11] This preparation was dissolved in distilled water and was sterilized in the autoclave at 15 pounds for 15 minutes. An extract of mouse organs (including thyroid, parathyroid, testis, kidney, liver and heart) was prepared by grinding the organs with water, heating to 50° C. for one hour and adding ethyl alcohol to a concentration of 80 per cent. The insoluble protein was filtered off and washed with water and the washings were added to the filtrate. This extract after adjustment to a pH of 7.3 and sterilization by Seitz filtration had a concentration of 40–50 mg./cc.

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[7] Stannard, J. N., *Am. J. Physiol.*, **122** (1938): 379; Saslow, G., *J. Cell. Comp. Physiol.*, **10** (1937): 385; Shorr, E., Barker, S. B., and Malam, M., *Science*, **87** (1938): 168; Barker, S. B., Shorr, E., and Malam, M., *J. Biol. Chem.*, **129** (1939): 33.

[8] Büngeler, W., *Frankfurt. Z. Path.*, **43** (1932): 409.

[9] Carroll, Sr. M. J., *Nature*, **143** (1939): 684.

A cell-free preparation from mouse embryos was prepared by grinding the embryos in sterile Ringer solution in the ratio of 1 cc. to each embryo. The embryo pulp was centrifuged off and the supernatant fluid was used without determination of its solid content.

The effects of the respiratory factors were tested on the following tissues: rat liver, mouse adenocarcinoma No. 63, a mouse sarcoma induced by the injection of methylcholanthrene, and a spontaneous rat sarcoma and transplants of it. The rat liver used was cut into slices approximately 0.3 mm. thick; the tumor tissues into small fragments. Into each test flask were placed 12–15 mg. (dry weight) of liver tissue and 5–6 mg. (dry weight) of tumor tissue.

The measurements of respiration and aerobic and anaerobic glycolysis were made by the indirect method of Warburg[12] using Ringer-bicarbonate-glucose (0.15 M with respect to bicarbonate, 0.2 per cent glucose, pH 7.3 approximately) as

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[10] Cook, E. S., Kreke, C. W., and Nutini, L. G., These Studies, 2 (1938): 23.

[11] Cook, E. S., and Walter, E. M., This Issue, p. 189.

[12] Warburg, O., *op. cit.*, p. 114.

TABLE I

Effect of "Crude" Respiratory Factor from Yeast on Respiration and Glycolysis of Rat Liver

Exp. No.	Q_{O_2}		$Q_G^{O_2}$		$Q_G^{N_2}$		$\frac{Q_G^{N_2}-Q_G^{O_2}}{Q_G^{N_2}}100$		$\frac{Q_G^{O_2}}{Q_{O_2}}100$	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
1	−7.57	−19.41	+3.48	0.0		+1.65		100	46	0.0
2a*	−8.25	−19.34	+0.85	0.0	+8.52	+2.98	90	100	10	0.0
2b	−8.78	−16.90	+2.06	0.0		+3.73		100	23	0.0
3a	−5.47	−17.96	+0.32	0.0	+3.43	+1.42	91	100	59	0.0
3b	−6.00	−17.34	+0.34	0.0		+1.23			56	0.0
4a	−5.18	−19.10	+0.54	0.0	+6.12	+2.27	91	100	10	0.0
4b	−6.04	−11.37	+1.01	0.0	+6.47		84		17	0.0
5a	−7.32	−16.13	+0.44	0.0		+1.64		100	60	0.0
5b	−7.03	−15.76	+1.17	0.0	+6.12	+1.68	81	100	17	0.0
6a	−6.53	−17.20	+0.86	0.0	+6.92	+2.10	88	100	13	0.0
6b	−8.80	−14.73	0.0	0.0	+5.61		100		0	0.0
7a	−8.62	−17.62	0.0	0.0	+3.42	+0.75	100	100	0	0.0
7b	−7.21	−29.17	0.0	0.0		+0.47		100	0	0.0
Average	−7.14	−17.85	+0.85	0.0	+5.83	+1.81				
	% Increase......150		% Decrease.....100		% Decrease......69					

The test material was the "crude" respiratory factor at a concentration of 8.20 mg./cc.

**a* and *b* represent duplicate determinations on tissue from the same animal.

TABLE II

Effect of Respiratory Factors on the Respiration and Glycolysis of Tumors

Exp.	Tissue	Test Material	Concentration	Q_{O_2}		$Q_G^{O_2}$		$Q_G^{N_2}$		$\frac{Q_G^{N_2}-Q_G^{O_2}}{Q_G^{N_2}}100$		$\frac{Q_G^{O_2}}{Q_{O_2}}100$	
				Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
1a*	mouse adenocarcinoma No. 63	embryo extract	1 cc./8 cc.	−9.06	−8.75	+7.55	+6.13	+34.56	+28.86	78	79	83	70
1b				−6.45	−7.76	+5.92	+5.12	+27.22	+38.51	78	81	81	66
2a				−11.92	−5.23	+9.89	+4.51	+36.53	+32.18	73	80	83	86
2b				−9.78	−10.24	+6.65	+6.28	+34.33	+30.38	81	79	68	61
Average				−9.30	−7.99	+7.50	+5.51	+33.16	+32.47	78	80	81	69
						% Decrease....27		% Decrease....2					
3a	mouse adenocarcinoma No. 63	embryo extract	3 cc./8 cc.	−2.24	−14.09	+7.98	+9.58	+33.60	+28.50	76	66	356	68
3b				−9.64	−17.76	+15.32	+9.46	+31.13	+33.18	51	71	159	53
4				−15.92	−27.15	+13.44	+13.58					84	50
Average				−9.27	−19.66	+12 28	+10.87	+32.36	+30.84	64	69	198	83
				% Increase .. 112		% Decrease....12		% Decrease.....5					
5a	mouse methylcholanthrene sarcoma	purified yeast extract	10.6 mg./cc.	−9.65	−13.63	+9.00	+7.16	+17.46	+13.27	48	46	93	53
5b				−15.42	−11.51	+11.68	+6.45	+16.12	+14.89	28	57	76	56
6a				−8.05	−11.36	+7.34	+6.60	+13.68	+12.92	46	49	91	58
6b				−2 77	−21.02	+9.33	+10.51	+10.31	+9.72	8	75	337	50
Average				−8.97	−14.38	+9.34	+7.68	+14.39	+12.70	33	58	109	53
				% Increase 60		% Decrease....18		% Decrease....12					
7	mouse methylcholanthrene sarcoma	purified yeast extract	13.7 mg./cc.	−1.58	−3.71	+2.42	+1.42	+4.30	+2.74	44	48	153	38
				% Increase ...135		% Decrease....41		% Decrease....36					

TABLE II—Continued

Exp.	Tissue	Test Material	Concentration	Q_{O_2}		$Q_G^{O_2}$		$Q_G^{N_2}$		$\frac{Q_G^{N_2} - Q_G^{O_2}}{Q_G^{N_2}} 100$		$\frac{Q_G^{O_2}}{Q_{O_2}} 100$	
				Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
8	rat spontaneous sarcoma	purified yeast extract	13.7 mg./cc.	−7.26	−25.77	+1.73	+0.71					24	3
9a				−6.41	−10.65	+4.08	+0.29		+4.04		93	64	3
9b				−4.86	−6.43	+4.38	+0.47	+10.80	+5.60	59	92	90	7
Average........................				−6.18 % Increase	−14.28 ...131	+3.40 % Decrease	+0.49 86	+10.80 % Decrease	+4.82 55			55	3
10a	rat transplanted spontaneous sarcoma†	purified yeast extract	10.6 mg./cc.	−0.29	−0.47	+0.59	0.00	+0.53	+1.27			201	0
10b				−0.33	−1.47	+0.88	+0.42	+1.32	+0.54	33	22	267	29
Average........................				−0.31 % Increase	−0.97 ...213	+0.73 % Decrease	+0.21 71	+0.92 % Decrease	+0.90 2			236	15
11a	mouse methylcholanthrene sarcoma	mouse organ extract No. 5 and 6	14 mg./cc.	−7.71	−22.20	+9.07	+3.55	+19.25	+5.79	53	38	118	16
11b				−8.09	−28.13	+9.19	+3.24					114	12
Average........................				−7.90 % Increase	−25.16 ...218	+9.13 % Decrease	+3.40 63	% Decrease	70			112	14

*a and b represent duplicate determinations on tissue from the same animal.

†This transplant contained a large amount of connective tissue and had a very low metabolism.

the medium. The technique and method of calculation of results are fully described by Warburg and need not be repeated here. The test materials, adjusted to pH 7.3, were added to the flasks in the concentrations shown in Tables I and II. Most of the experiments extended over a period of 1 hour. In some cases where only a small amount of tumor tissue was available the time was prolonged to 2 hours. The rates of respiration appeared to remain constant over a 2-hour period so that results obtained from both periods were comparable.

DISCUSSION

The results are shown in Tables I and II. In these tables Q_{O_2} represents the oxygen uptake in cubic millimeters per milligram of dry weight of tissue per hour. $Q^{O_2}_{G}$ and $Q^{N_2}_{G}$ represent the number of cubic millimeters of lactic acid produced aerobically and anaerobically, respectively, per milligram of dry weight of tissue per hour. These values, of course, are expressed in terms of the equivalent amounts of CO_2 produced from bicarbonate by the lactic acid (1 cu. mm. CO_2 being equivalent to 0.004 mg. lactic acid). The final two columns in each table represent, respectively, the percentage effectiveness of the Pasteur reaction and the ratio of aerobic glycolysis to respiration.

From Table I it will be seen that the crude respiratory factor from yeast in the concentration employed caused a large increase in oxygen uptake by rat liver. At the same time it caused a 69 per cent inhibition of anaerobic glycolysis and complete suppression of aerobic glycolysis. Under normal conditions liver has a high respiration with a very low, or, in some experiments, no aerobic glycolysis. This, in itself, suggests the independence of the glycolytic and respiratory processes. It will be observed that under these conditions the Pasteur reaction was from 80 to 100 per cent operative and always became 100 per cent effective after addition of the yeast extract.

Table II shows that a purified yeast preparation stimulated the respiration of mouse methylcholanthrene sarcoma and of the spontaneous rat sarcoma. The same extract caused a depression of aerobic and anaerobic glycolysis in the same tumors. These results are of interest in the light of those of Carroll[9] referred to earlier.

The mouse embryo extract in the lowest concentration studied had only a slight effect on the respiration and glycolysis of adenocarcinoma No. 63. However, when the concentration was increased a marked stimulating effect was observed on the respiration of this tumor, but the depressing effect on glycolysis was still not very marked.

The extract of mouse organs, in the two experiments reported, was notably effective in stimulating the respiration of the methylcholanthrene sarcoma and in depressing both aerobic and anaerobic glycolysis. This preparation was free of protein and thus confirms the observation of Büngeler[8] that protein does not play a part in the observed effects.

All of the factors tested in active concentrations increased the respiration and decreased, to a variable degree, both aerobic and anaerobic glycolysis of all tumors studied. Great quantitative variation between different tumors was evident, as would be expected, but in every case the ratio of aerobic glycolysis to respiration was reduced by the addition of the respiratory factors. It may be noted, however, that only the yeast extracts were significantly successful in increasing the effectiveness of the Pasteur reaction and this, frequently, to no great extent. In the other cases there was no significant increase and in certain cases (for which, however, insufficient data are available for satisfactory conclusions) the Pasteur effect seems actually to be inhibited. Thus, in certain instances, at least, the extracts seem to act by independently increasing the respiration and depressing the aerobic and anaerobic glycolytic processes and not by increasing the effectiveness of oxygen in suppressing glycolysis. Büngeler[8] found spleen extracts to increase the Pasteur effect. It is of interest that in the authors' experiments the Pasteur effect appears to be normally operative to a greater degree with carcinoma No. 63 than with the other tumors studied.

In these laboratories[13] it has been shown that fractions (including those free of protein) prepared from beef spleens will stimulate the respiration of certain tumors as well as of normal tissues. This is of particular interest in view of Lewisohn's ability to cause the retrogression of mouse sarcoma No. 180 with beef spleen extract.[14] The papers cited also contain an account of the effects on other tissues of some of the extracts studied in the present paper.

The work of Büngeler and of Carroll has already been referred to. Their experiments, as well as those of the authors and of others,[13] show that it is possible to alter the characteristics of tumor metabolism *in vitro* by cell-free tissue extracts. It is of interest that Büngeler found spleen extracts to be more effective on tumor metabolism than on that of normal tissues. The authors have found great variation in the relative effectiveness of different extracts on various tissues. It will be of particular interest to correlate the metabolic effects of these extracts with their power to immunize against tumor, or to cause tumor regression. Experiments of this type are in progress in these laboratories.

The experiments reported in this paper may lend support to the idea that the glycolytic steps do not precede respiration, i.e., that lactic acid need not first be formed and then oxidized.

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[13] Fardon, J. C., Brotzge, G. C., and Loeffler, Sr. M. K., THESE STUDIES, in preparation; Cook, E. S., Walter, E. M., and Sacksteder, V., *ibid.*, in preparation.

[14] Lewisohn, R., *Surg. Gynecol. Obstet.*, 66 (1938): 563.

Since the cellular extracts studied depress both anaerobic and aerobic glycolysis and stimulate respiration it would seem unlikely that glycolysis must precede respiration. It must be pointed out that inhibition of anaerobic glycolysis does not approach completion, as may be the case with iodoacetate, although a 69 per cent inhibition was encountered for liver.

Before applying these findings to the nature of the Pasteur reaction, however, it is essential that we know more of the mechanism by which these respiratory factors act. It is, of course, essential that the respiratory effects be referable to carbohydrate and not to the oxidation of other substances. For example, it is not inconceivable that certain materials in these extracts may act as glycolytic inhibitors and at the same time that these materials or others present in the extracts may furnish oxidizable non-carbohydrate substrates or may stimulate non-carbohydrate oxidation, although an abundance of carbohydrate was present in the medium. It should be possible to throw light on these problems by studying the effects of the respiratory-stimulating factors on the respiratory quotient. Such studies have been begun. The question of substrate in tumor respiration has been raised by Dickens[15] and by Elliott.[16] These studies will also be extended to other tissues, such as brain, which can utilize carbohydrates exclusively.

SUMMARY

Extracts from yeast and animal tissues have been shown to increase the respiration and to depress both the aerobic and anaerobic glycolysis of normal (liver) and tumor tissues (mouse adenocarcinoma No. 63, mouse methylcholanthrene sarcoma, spontaneous rat sarcoma). There is a probable relation of these findings to tumor metabolism and the Pasteur reaction.

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[15] Dickens, F., and Simer, F., *Biochem. J.*, 24 (1930): 1301; 25 (1931): 985.

[16] Elliott, K. A. C., and Baker, Z., *Biochem. J.*, 29 (1935): 2433.

LITERATURE CITED

Bancroft, G., Beck, L. V., and Russell, M. A., *Biochem. J.*, 29 (1935): 2416.

Barker, S. B., Shorr, E., and Malam, M., *J. Biol. Chem.*, 129 (1939): 33.

Boyland, E., and Boyland, M. E., *Biochem. J.*, 33 (1939): 618.

Büngeler, W., *Frankfurt. Z. Path.*, 39 (1930): 314; 43 (1932): 409.

Burk, D., in Some Fundamental Aspects of the Cancer Problem, Science Press, New York, 1937, p. 121.

Carroll, Sr. M. J., *Nature*, 143 (1939): 684.

Cook, E. S., Kreke, C. W., and Nutini, L. G., THESE STUDIES, 2 (1938): 23.

Cook, E. S., and Walter, E. M., THIS ISSUE, p. 189.

Crabtree, H. G., *Biochem. J.*, 23 (1929): 536.

Davis, J. E., *Can. Med. Assoc. J.*, 36 (1937): 27.

Dickens, F., and Simer, F., *Biochem. J.*, 24 (1930): 1301; 25 (1931): 985.

Dixon, K. C., *Biol. Rev.*, 12 (1937): 431.

Elliott, K. A. C., and Baker, Z., *Biochem. J.*, 29 (1935): 2433.

Fardon, J. C., and Sullivan, W. A., *Nature*, 143 (1939): 287.

Fardon, J. C., Sullivan, W. A., Brotzge, G. C., Loeffler, Sr. M. K., and Andrus, Sr. M. B., THIS ISSUE, p. 203.

Lewisohn, R., *Surg. Gynecol. Obstet.*, 66 (1938): 563.

Murphy, J. B., and Hawkins, J. A., *J. Gen. Physiol.*, 8 (1925): 115.

Saslow, G., *J. Cell. Comp. Physiol.*, 10 (1937): 385.

Shorr, E., Barker, S. B., and Malam, M., *Science*, 87 (1938): 168.

Stannard, J. N., *Am. J. Physiol.*, 122 (1938): 379.

Warburg, O. (Trans. by F. Dickens), The Metabolism of Tumours, Constable, London, 1930.

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BENDING AND CELL ENLARGEMENT IN THE HYPOCOTYL OF HELIANTHUS ANNUUS

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RECENT discoveries on the nature and action of biochemical regulators and developments in the study of tropisms have stimulated the investigation of growth-promoting substances.* The rôle of auxins as growth promoters in plants has been demonstrated by a number of investigators.[1] Ordinarily the term "growth" is taken to mean a proliferation of cells, but it may also mean the mere enlargement of cells. Whatever rôle bios may play in the growth of the cell, evidence seems to point to the fact that auxin produces or induces cell enlargement.[2]

In previous experiments the authors showed by direct measurement that the cells of different regions of the hypocotyl of *Helianthus annuus* are not of the same size.[3] The cells nearer the cotyledons are considerably smaller than those farther removed from them. The mass of the protoplasm of the larger cells is not appreciably greater than in the smaller cells, but the amount of solution stored in the cell-sap cavity of the larger cells is partially, if not wholly, responsible for their greater size.

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*When animal cells are injured growth-promoting factors are liberated which stimulate cell proliferation (Ref. Fardon and Sullivan, THESE STUDIES, 2 (1938): 39; Loofbourow, Dwyer and Morgan, *ibid.*, p. 137).

[1] (a) Boysen-Jensen, P., Translated by Avery, G. S., and Burkholder, P. R., Growth Hormones in Plants, McGraw-Hill, New York, 1936.

(b) Went, F. W., and Thimann, K. V., Phytohormones, Macmillan Co., New York, 1937.

[2] Heyn, A. N. J., *Protoplasma*, 19 (1933): 78.

[3] Beck, W. A., Schocken, K., and Donnelly, Sr. M. W., THESE STUDIES, 2 (1938): 107.

It seems clear that the amount of solution produced in a cell increases with its age. The concentration of the sap in the older cells need not necessarily be greater or less than in the younger cells. The osmotic value of the cells, which is the osmotic pressure of the cell sap in the state of incipient plasmolysis, of a given tissue is not the same for all the cells but the osmotic value of none of them varies greatly from the average value for the tissue.

For the corticle tissue of the region between 0 and 5 mm. below the cotyledons it was 0.149 mol ($C_{12}H_{22}O_{11}$) while the average at a distance between 45 and 50 mm. below the cotyledons (where the cells were considerably enlarged) the O_g was 0.148 mol ($C_{12}H_{22}O_{11}$). In a similar manner the O_g for the epidermal cells was found to be 0.225 mol ($C_{12}H_{22}O_{11}$) at 5 mm. below the cotyledons and 0.200 mol ($C_{12}H_{22}O_{11}$) at 45 to 50 mm. As the cells mature they can simultaneously increase the amount of solute and absorb an equivalent quantity of water and adjust the cell-wall deposit so that the capacity of the cell (for cell sap) is increased without changing the osmotic value of the cell, the hydrostatic pressure, the wall pressure or the suction tension. To avoid confusion we shall use the term "hydrostatic pressure" as the pressure produced in a cell by the influx of water into the cell-sap cavity; "wall pressure" as the pressure in the cell wall due to stretching, which is antagonistic to the hydrostatic pressure;[4] "suction tension" the positive or negative pressure per unit area which causes the influx or efflux of water through the cell wall, and whose numerical value is the difference between the osmotic pressure of the cell sap and the wall pressure.[5]

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[4] Ursprung, A., and Blum, G., *Ber. deut. botan. Ges.*, 34 (1916): 525.

[5] (a) Beck, W. A., *Plant Physiol.*, 3 (1928): 413.

(b) Ursprung, A., and Blum, G., *Biol. Zentr.*, 40 (1920): 193.

(c) Ursprung, A., *Plant Physiol.*, 10 (1935): 115.

Such a development of the cell can properly be called a growth since it involves the metabolic processes of the protoplasm and the increment of cell products (solute and cell-wall material).[1a, 6] The mechanism involved in the process of enlargement is not clearly understood. The enlargement of the cell is not merely the effect of the turgor produced by the influx of water. De Vries[7] and Schwendener and Krabbe[8] attempted to determine the turgor strain of the cell wall by means of the elastic contraction, which is produced at incipient plasmolysis, assuming that the stretching force is proportional to the strain. Ursprung and Blum[9] showed that the turgor is not proportional to cell enlargement.* Conceivably the turgor might enlarge the cell and stretch the wall, keeping the protoplasm in constant contact with the wall. As the wall is stretched new material may be added by the protoplasm in such a manner that its elastic constants are maintained.[10] The recent works of Bailey,[11] Anderson and Kerr[12] and Wergin[13] cite results in harmony with this view.

Some unpublished investigations of these laboratories on the osmotic value of cells in the hypocotyl of *Helianthus annuus* show that the cell walls of older cells fail to return to the

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[6](a) Thimann, K. V., and Bonner, J., *Proc. Roy. Soc.*, London, B., 113 (1933): 126.

(b) Heyn, A. N. J., *Rec. trav. botan. néerland.*, 28 (1931): 113.

[7]Vries, H. de, *Jahrb. wiss. Botan.*, 14 (1884): 427.

[8]Schwendener, S., and Krabbe, G., *Jahrb. wiss. Botan.*, 25 (1893): 35.

[9]Ursprung, A., and Blum, G., *Jahrb. wiss. Botan.*, 63 (1924): 1.

*"Die Versuche, . . . ergaben übereinstimmend, dass auf diesem Wege zwischen Turgordehnung und Längenwachstum keine Proportionalität nachweisbar ist." (p. 65).

[10]Pfeffer, W., Pflanzenphysiologie: Ein Handbuch der Lehre von Stoffwechsel und Kraftwechsel in der Pflanze, Bd. II, Kraftwechsel, W. Engelmann, Leipzig, 1881, 2nd. ed., 1904.

[11]Bailey, I. W., *Ind. Eng. Chem.*, 30 (1938): 40.

[12]Anderson, D. B., and Kerr, T., *Ind. Eng. Chem.*, 30 (1938): 48.

[13]Wergin, W., *Angew. Chem.*, 49 (1936): 843; Kögel, F., *Naturwissenschaften*, 25 (1937): 465.

position in which they were when the cells were younger. It might be supposed that this failure to return to the original position when the hydrostatic pressure is relieved, is due to stretching the wall beyond the limits of elasticity. Ursprung and Blum[9] claimed that the limits of elasticity were never exceeded when the cell walls in the root tips of *Vicia Faba* were stretched by the turgor.†

It would appear from the results that Pfeffer's notion is correct, namely, that cell wall material is constantly formed and regularly deposited in the wall during the process of cell enlargement. According to the recent studies of Buck[14] the results of Ursprung and Blum may be criticized. Ursprung and Blum worked with normal tissue in which the turgor remained, while Buck worked with tissue in which the hydrostatic pressure was released. Buck concluded that the cell wall is plastic in older as well as in younger cells but that it is more plastic in the younger. It is true, however, that Ursprung and Blum recognized the fact that in young and growing cells the modulus of elasticity of the wall was less than in older cells.[9]* They recognized, furthermore, that the tissues of the growing zones were more plastic than the older tissues.[9]‡ Buck also concluded that the micells are evidently dislocated under the influence of hydrostatic pressure. From this it is evident that the wall pressure cannot be proportional to the influx of water in growing cells for any length of time.

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† "Eine bleibende Verlängerung ist auch hier nicht vorhanden, und so liessen denn alle diese Versuche in der wachsenden Wurzelspitze unserer *Vicia Faba* eine Überschreitung der Elastizitätsgrenze der Wand durch Turgordehnung nicht erkennen." (p. 65).

[14] Buck, L., *Botan. Centr. Beihefte, A.*, 53 (1935): 340.

* "So ziehen wir also aus dem Versuchsresultat den Schluss, dass das grosse in der Umgebung des Streckungsmaximums durch ein Minimum des Elastizitätsmodulus bezw. ein Maximum des Dehnungskoeffizienten bedingt ist." (p. 60).

‡ "Wir schliessen daraus, dass die Zellwände in der Umgebung des Streckungsmaximums auch die grösste Plastizität besitzen." (p. 60).

There can hardly be doubt that the cell wall is elastic to some degree even though the value of the "yield point" is not very great, since simple experiments of plasmolysis such as de Vries[7] carried out, show evident contraction of the cell after the hydrostatic pressure is released. It seems clear that in this process of cell enlargement, cell-wall material and solute (which appear in the cell-sap cavity) are produced simultaneously by the metabolic processes of the cytoplasm. This is done with the probable co-operation of the nucleus and in such a manner that the capacity of the cell (for containing solution) is constantly increased, without considerable variation in the osmotic value of the cell, the wall pressure, or the suction tension of the cell, in spite of the constant influx of water into the cell-sap cavity. This process of cell enlargement must consequently be considered a true growth involving the metabolic activity of the protoplasm.

The authors' experience in the study of the osmotic relations of cells and their relative sizes in various parts of the hypocotyl of 90-hour-old Helianthus seedlings is in harmony with this view. Furthermore, it is clear from their preliminary experiments that this process of enlargement does not go on indefinitely. There is a critical point beyond which enlargement ceases, or at least is considerably reduced. The cessation of enlargement may be due to the exhaustion of growth-promoting substances within the protoplasm of the cell, the diminution of the supply of growth-promoting substances from without the cell, or to the production of some growth-inhibiting substance within or without the cell. Went[15] has shown that other hormone-like factors (calines) are required for cell elongation.

It is reasonable to assume that the nutrients stored in the cotyledons are necessary for this mode of growth. It also appears evident that growth promoters, such as auxins, are involved in the process.

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[15] Went, F. A., *Plant Physiol.*, 13 (1938): 55.

TABLE I

The Angle of Bending and the Critical Point of Helianthus Hypocotyls under the Influence of Gravity

Experiment	Number of Plants Used	Hours of Exposure	Condition of Plants	Average Length of Hypocotyl in cm.	Angle of Bending in Degrees	Critical Point below the Cotyledons in cm.
No. 1..............	14	2	With Cotyledons	3.62	29.0	2.15
No. 2..............	14	4	With Cotyledons	3.37	47.3	2.20
No. 3..............	14	2	Cotyledons removed	3.10	25.8	2.14
No. 4..............	14	4	Cotyledons removed	3.42	44.7	2.66

In these experiments the authors were interested in finding at which particular point on the hypocotyl the cells became mature, that is, at which point in the stage of development the enlargement of the cells failed to proceed at an appreciable speed. The known relation between auxin stimulation and geotropism offered a means of studying the response of cells in different regions of the hypocotyl.

METHODS

The seedlings employed were grown in the dark chamber because auxin production is seriously affected by light.[10, 16] The temperature was maintained at approximately 30° C. Provision was made to maintain 90 to 100 per cent relative humidity in the sprouting chamber. The plants were grown in small pots containing a mixture of equal parts of sand and German peat, to which Knop's solution was added. All seedlings used were 90 hours old at the beginning of each experiment. In most of the experiments 7 seedlings were planted in each pot. The cotyledons of these were not uniformly directed. In later experiments, only one seed was planted in each pot, which permitted the cotyledons to be directed at will in a given experiment.

EXPERIMENTAL

SERIES I

Critical point and origin of growth-promoting substances

Besides determining the critical point of cell enlargement in this series of experiments an effort was made to learn if the cotyledons were a source of growth-promoting substances. Four sets of experiments were performed and are recorded in Table I. The control plants are not listed because in all cases

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[16] Fliry, M., *Jahrb. wiss. Botan.*, 77 (1932): 150.

they were strictly orthotropus. In one set of test plants the cotyledons remained intact while in others they were removed. Some test plants were exposed to the geotropic influence for 2 hours and others for 4 hours. In Table I the average length of the seedlings is given because the length may be a factor in auxin production.[17]

There was no great variation in the average size of the plants because practically constant conditions were maintained during the sprouting period. The experimental plants were taken from the dark chamber and placed in another dark chamber in which the temperature and relative humidity were the same. They were subjected to geotropic influence by so placing the pots that the hypocotyl was horizontal, leaving the cotyledons of some of the plants in a dorsiventral position, and others at random. The relative position of the cotyledons could have some bearing on the influence of gravity and on auxin translocation. In the first and second sets of plants the cotyledons were not removed; in the third and fourth sets they were removed. In the first and third sets the experimental time was 2 hours; in the second and fourth sets it was 4 hours.

DISCUSSION

The degree of bending was greater when the experimental time was greater, both with and without the cotyledons. The normal degree of bending was somewhat inhibited by the removal of the cotyledons. The difference, however, was not great. When the experimental period was 2 hours the difference was 3.2° (29°−25.8°) and when it was 4 hours the difference was 2.6° (47.3°−44.7°). It might have been that this slight difference was caused by a lack of nutrient material which should normally have been derived from the excised cotyledons.[15] Since the difference in the effect produced with and without the cotyledons is not great even during an exposure of 4 hours, it seems improbable that there was insufficient stimulating material. It would rather seem that the condition of the cell walls must be held responsible for the lack of response in the older cells.

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[17] Dijkman, M. J., *Rec. trav. botan. néerland.*, 31 (1934): 391.

From the table it is evident that increasing the time of exposure from 2 to 4 hours did not materially change the point of deviation below the cotyledons from the orthotropus condition. The point of deviation appears to mark the limit at which cells can still be affected by the influencing factors. The fact that the cells beyond the point of deviation fail to respond geotropically even after 4 hours exposure may indicate that the cells can no longer be affected by the phytohormone. In general, it may be supposed that cells can be stimulated without being able to respond, due to some impeding mechanical factor. In the authors' experiments there did not seem to be a lack of the phytohormone since the degree of bending continued to increase. If the lack of nutrient is made responsible for the cessation of progressive response in the basipetal direction, then it is to be expected that a decided difference between the degree of bending be shown in the experiments (1 and 2) in which the cotyledons were intact and in those (3 and 4) in which the cotyledons were removed. The actual differences were favorable to such a conclusion but they were very small (29° – 25.8° and 47.3° – 44.7°). It may be that the lag in response is due to an increased resistance to the conduction of the stimulating material (auxin) in the more mature cells. A reduced rate of translocation would cause an insufficient amount of growth-promoting substance to be supplied. It may also be true that older and more sluggish cells require larger amounts of stimulating material to produce an appreciable response.

Comparing the distance of the point of deviation from the tip in Experiment 1 with the distance of the point in Experiment 3, and Experiment 2 with Experiment 4, it becomes evident that the removal of the cotyledons did not seriously affect the distance from the cotyledons of the point marking the limit of sensitivity. This would indicate that the cells which fail to respond to stimulation are not affected by the removal of the source of nutrient and possible source of growth-promoting substance. This same argument holds if we make the plumule responsible for the supply of growth-promoting substance and the cotyledons the source of nutrients.[18]

CONCLUSIONS

From the data and the discussion given above it is evident that there is a critical point on the hypocotyl where the cells no longer enlarge or at least do not enlarge at an appreciable rate. The failure to enlarge appears to be due to the condition of the older cells rather than a lack of nutrient or stimulating material.

Since the removal of the cotyledons did not materially affect either the angle of bending or the distance of the critical point from the cotyledons, it appears evident that the cotyledons are not the sole source of the growth-promoting substance. The fact that geotropic bending occurs after the removal of the cotyledons, favors the view that the growth-promoting substance is present throughout the growing region. This agrees with the findings of Herzog.[18]

It is possible that the hypocotyl contained enough nutrient and growth-promoting substance in the process of translocation, that the negative effect of the removal of the cotyledons would not become manifest during the period of exposure. The possibility is emphasized by the fact that whether or not the cotyledons were removed the angle of bending was greater after 4 hours exposure than after only 2 hours. If the results are ascribed to the presence of a growth-promoting substance

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[18] Herzog, W., *Planta*, 1 (1925): 116.

TABLE II

The Angle of Bending and the Critical Point of Helianthus Hypocotyls under the Influence of Gravity

Experiment	Number of Plants Used	Hours of Exposure	Condition of Plants	Average Length of Hypocotyl in cm.	Angle of Bending in Degrees	Critical Point below the Cotyledons in cm.
No. 5..............	14	2	One cotyledon removed	2.9	38.0	2.30
No. 6..............	14	4	One cotyledon removed	2.9	40.4	2.71
No. 7..............	14	2	Upper half of each cotyledon removed	2.8	33.6	2.57
No. 8..............	14	4	Upper half of each cotyledon removed	2.8	43.6	2.77

in the hypocotyl, then it is evident that it was not exhausted after 2 hours of activity in spite of the lack of a fresh supply of the promoter itself (or a precursor) from the cotyledons.

SERIES II

Distribution of growth-promoting substance in the cotyledons

Another series of experiments was carried out to discover the effects of the removal of one cotyledon and the removal of the upper half of each cotyledon. The general procedure, the age of the seedlings, the temperature and the relative humidity were the same as in the previous series of experiments. Control plants were used but are not listed in the table for reasons stated previously (cf. p. 265). The results are recorded in Table II.

DISCUSSION AND CONCLUSIONS

Comparing Tables I and II we find the values recorded of the same order. The angle of bending during the initial 2 hours was somewhat greater in this series but the final value at the end of 4 hours was approximately the same. The results strengthen the conclusions reached in the previous series of experiments. In particular, if growth-promoting substances and the nutrients required for cell enlargement are located in the cotyledons, they must be evenly distributed there because whether one of the cotyledons was entirely removed or merely the upper half of each made no appreciable difference in the degree of bending or in the distance of the critical point from the base of the cotyledons.

SERIES III

The effect of eliminating portions of the hypocotyl

The next experiments investigated the effect of removing portions of the hypocotyl between the critical point and the

TABLE III

Critical Point in the Hypocotyl of Helianthus Annuus

Experiment	Number of Plants Used	Hours of Exposure	Portion of Hypocotyl Removed in cm.	Critical Point in cm. from the base of the Cotyledons
No. 9	14	3	0.5	2.2
	14	4	0.5	2.6
	14	6	0.5	1.8
	14	8	0.5	2.9
	14	16	0.5	2.7
No. 10	14	3	1.0	1.2
	14	4	1.0	2.2
	14	6	1.0	2.3
	14	8	1.0	2.8
	14	16	1.0	3.1
No. 11	14	3	1.5	2.2
	14	4	1.5	2.2
	14	6	1.5	2.2
	14	8	1.5	1.5
	14	16	1.5	3.4
No. 12	14	3	2.0	2.0
	14	4	2.0	2.0
	14	6	2.0	2.0
	14	8	2.0	2.0
	14	16	2.0	2.0

base of the cotyledons. It seemed possible that the critical point depends upon nutrients or growth-promoting substances in the course of translocation or it might depend upon those permanently lodged in the region of the younger cells.

The general procedure was the same as in the previous experiments except that the upper portions of the hypocotyl were removed and the time of exposure was varied. The results are recorded in Table III.

TABLE IV

Critical Point on the Hypocotyl of Helianthus Annuus

Experiment	Number of Plants Used	Average Length of Hypocotyl in cm.	Minutes of Exposure	Condition of Plants	Distance of the Critical Point below the Cotyledons in cm.
No. 13	14	3.0	15	With Cotyledons	1.50
No. 14	14		30	With Cotyledons	1.60
No. 15	14		45	With Cotyledons	1.73
No. 16	14		60	With Cotyledons	2.08
No. 17	14	3.0	15	All Cotyledons removed	1.57
No. 18	14		30	All Cotyledons removed	1.66
No. 19	14		45	All Cotyledons removed	1.84
No. 20	14		60	All Cotyledons removed	2.30
No. 21	14	2.9	15	One Cotyledon removed	1.53
No. 22	14		30	One Cotyledon removed	1.60
No. 23	14		45	One Cotyledon removed	2.43
No. 24	14		60	One Cotyledon removed	2.50
No. 25	14	3.1	15	Upper half of each Cotyledon removed	1.60
No. 26	14		30	Upper half of each Cotyledon removed	1.50
No. 27	14		45	Upper half of each Cotyledon removed	2.05
No. 28	14		60	Upper half of each Cotyledon removed	2.05

DISCUSSION AND CONCLUSIONS

In spite of the removal of the uppermost parts of the hypocotyl, the position of the critical point varied but little from its position in the previous experiments, when these parts were retained, nor did longer exposure materially change the position of the critical point. Hence, we are forced to the conclusion that sufficient nutrient and stimulating material are present to affect cells which are still susceptible. Furthermore, it seems evident that older cells are in a mature state and can no longer be considerably enlarged by growth-promoting substances and nutrients. It is surprising how sharply defined is the point of demarcation between the immature and mature cells. This point is approximately 2.5 cm. below the cotyledons.

SERIES IV

The speed of response

This series of experiments was conducted to determine the time required for the cells to be affected by growth-promoting substances. Readings were taken every 15 minutes for a period of 1 hour. In the first set the cotyledons were left intact; in the second, both cotyledons, including the plumule, were removed; in the third, only one cotyledon was removed; and in the fourth, only the upper half of each cotyledon was removed. All the plants were kept in the dark during the course of the experiments. Plants once exposed to light during the period of measurement were discarded; otherwise the general procedure was the same as in the previous experiments. The results are assembled in Table IV. The average length of the plants was approximately the same. In the table the average length is given only for Experiments 13, 17, 21 and 25 because time would not permit the measurement of all the plants.

DISCUSSION AND CONCLUSIONS

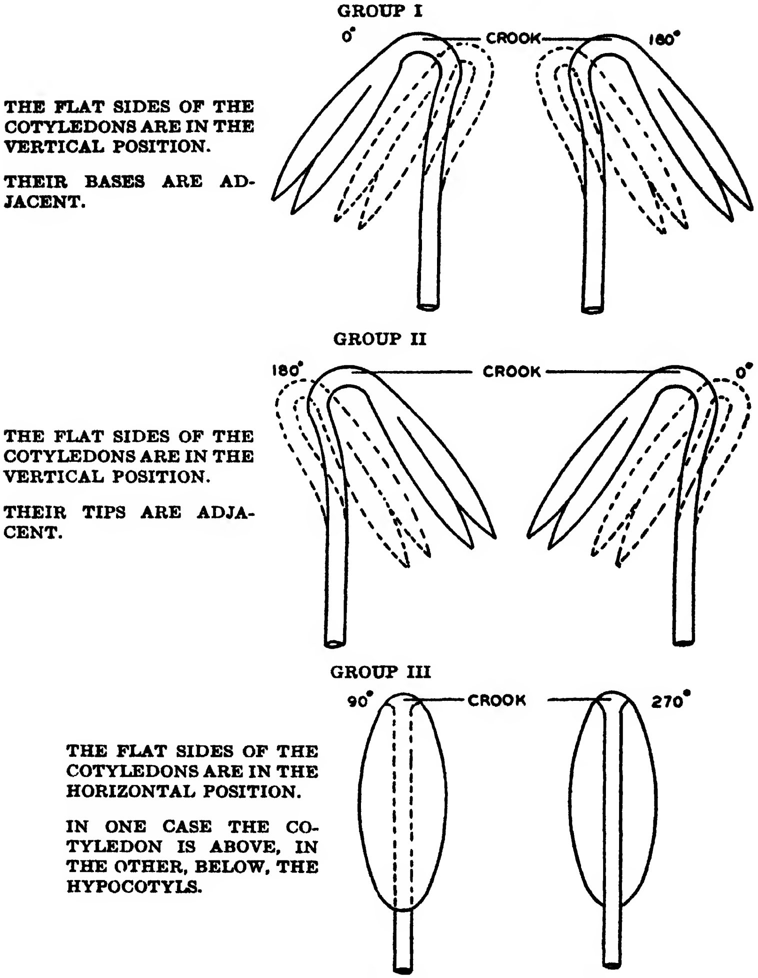
These as well as previous experiments make it evident that the removal of the cotyledons or portions of the cotyledons does not affect the point of deviation from the orthotropus position after a given period of exposure. It is apparent that the point of deviation does not depend upon a supply of nutrient or growth-promoting substance emanating from the cotyledons or the plumule. From Table IV it is clearly apparent that the cells at the critical point are affected even after 60 minutes of exposure. The rate of progress in a given period of time of the point of deviation from the orthotropus position is not constant. Within the first 15 minutes the point of bending was at a distance of 1.5 cm. below the cotyledons, during the next 15 minutes the point had progressed only 1 mm. This approximate rate (1 mm. per 15 minutes) is maintained in the succeeding two periods of exposure. The very young cells do not appear to be greatly affected by the growth-promoting substance. At least no great difference between the rate of growth on the upper side of the horizontal hypocotyl and the lower side could be detected in the form of bending, but a more careful study of the behavior of the cells of this region was made in the next series of experiments. Apparently the younger cells are affected more rapidly than the older cells lying nearer the critical point and, as the previous experiments showed, those lying beyond the critical point are not affected to any appreciable extent.

SERIES V

An unusual bending of the hypocotyl

In this series the authors studied a peculiar bending observed in the hypocotyl that could not be ascribed to geotropism. It seemed evident in some preliminary experiments that the direction of bending is somewhat dependent on the position

FIG. 1—*Unusual Bending of Hypocotyls*



THE FLAT SIDES OF THE COTYLEDONS ARE IN THE VERTICAL POSITION.

THEIR BASES ARE ADJACENT.

THE FLAT SIDES OF THE COTYLEDONS ARE IN THE VERTICAL POSITION.

THEIR TIPS ARE ADJACENT.

THE FLAT SIDES OF THE COTYLEDONS ARE IN THE HORIZONTAL POSITION.

IN ONE CASE THE COTYLEDON IS ABOVE, IN THE OTHER, BELOW, THE HYPOCOTYLS.

Drawing showing the relative positions of the cotyledons and the hypocotyl in three groups of plants used in the experiments of Series V. The full-line drawing represents the original positions, the dotted lines represent the succeeding positions. In Group III the succeeding positions could not easily be indicated. In the 90° position the plants moved downward while in the 270° position they moved upward. In all cases the Hypocotyls are in the horizontal position.

TABLE V

The Record of Unusual Bending of the Hypocotyl of Helianthus

Time of Exposure in Minutes	Relative Positions of the Cotyledons*					
	Distance of the Point of Deviation in mm.					
	Group I		Group II		Group III	
	Position		Position		Position	
	0° a	180° b	180° a′	0° b′	90° a″	270° b″
5	0	0	0	0	0	0
10	1 R	-	1 L	1 R	1 D	2 U
15	3 R	-	3 L	4 R	3 D	4 U
20	5 R	-	4 L	6 R	5 D	7 U
25	8 R	-	7 L	9 R	5 D	9 U
30	11 R	-	10 L	11 R	5 D	12 U
35	13 R	-	13 L	12 R	5 D	15 U
40	16 R	-	15 L	15 R	3 D	18 U
45	18 R	-	16 L	16 R	2 D	20 U
50	20 R	-	18 L	17 R	2 D	21 U
55	21 R	-	20 L	19 R	1 D	22 U
60	23 R	-	20 L	20 R	0	22 U
65	23 R	-	20 L	20 R	0	24 U
70	24 R	-	20 L	20 R	2 U	24 U
75	24 R	-	20 L	20 R	3 U	25 U
80	24 R	-	20 L	20 R	5 U	25 U
85	24 R	-	20 L	20 R	5 U	25 U
90	24 R	-	20 L	20 R	7 U	25 U
95	24 R	-	20 L	20 R	7 U	25 U
100	24 R	-	20 L	20 R	9 U	25 U
105	25 R	-	21 L	20 R	9 U	25 U
110	25 R	-	21 L	20 R	9 U	25 U
115	25 R	-	21 L	20 R	10 U	25 U
120	25 R	-	21 L	20 R	10 U	25 U

*All hypocotyls were in a horizontal position.

R—Right; L—Left; D—Down; U—Up.

of the cotyledons. This bending affected the readings of the degree of bending ascribed to geotropism and it was therefore more closely investigated in these experiments.

Six sprouts, planted singly in pots, were used. The testa was removed from each pair of cotyledons. Since the seeds were planted in a mixture of sand and peat the soil offered little resistance to the shift in position of the growing seed and as a consequence the testa was invariably carried, like a cap, on the cotyledons above the soil. The pair of cotyledons was bent to one side of the hypocotyl, like a crook. In all the tests the hypocotyls were in the horizontal position. The work was done in a dark room using only a safety lamp during the operation. The plants were arranged in three groups as illustrated in Fig. 1. In the first group a pair of plants was so placed that the flat sides of the cotyledons were in the vertical position and the crooks pointed away from each other; in the second group the flat sides of the cotyledons were again in the vertical position but the crooks turned toward each other; in the third group the flat sides of the cotyledons were in the horizontal position and in one case the crook pointed upward and in the other downward. The results are recorded in Table V.

DISCUSSION AND CONCLUSIONS

From the results obtained in Table V it became immediately evident that there was a decided bending which could not be regarded as geotropic but was independent of the position of the plants. The direction of the deviation, always opposite to the position of the cotyledons, was such that it would normally tend to move the cotyledons from the testa if it remained firmly lodged in the soil. It was surprising that this effect should be produced even after the removal of the testa and would indicate that the influencing factor is not in the testa itself.

The position of the plants in Groups I and II was the same, with the exception that the two plants in Group I had the crooks turned away from each other, while in Group II they were turned toward each other. No mutual inductive effect from the relative position of the cotyledons in Groups I and II could be observed. The figures given in column *a* for Group I are comparable with the figures in column *b′* Group II. The speed and degree of bending are almost the same. In both cases the bending was in the direction opposite the position of the cotyledons. It was practically impossible to determine precisely when the lateral bending ceased and the negatively geotropic bending began. The two movements merged in such a manner that a solid curve was produced. After approximately 45 minutes of exposure the lateral bending was very much reduced (from 45 minutes to 120 minutes the increment was not more than 6 mm.). Plant *b* in Group I, for some unaccountable reason, behaved abnormally. It failed to bend and the two cotyledons rapidly turned away from each other without causing the crook to bend either to the right or left. Plant *a′* behaved normally and the results are comparable with those recorded for *a* and *b′*. Again the degree of bending was of the same order as for the latter two plants, but the direction of bending was opposite. In Group III one plant moved upward and the other downward, with a bending similar to that observed in Groups I and II.

GENERAL RESULTS AND CONCLUSIONS

In Series I a study of the point of bending made it evident that there is a critical point on the hypocotyl (2.5 cm. below the cotyledons) beyond which no bending occurs in response to gravity. The effect of gravity was first made evident on the younger cells, a short distance beneath the cotyledons, and little by little, the effect was observed in the older cells in the order of their age.

The youngest cells (very close to the cotyledons), manifested a response to some unknown influence which tended to bend the hypocotyl away from the base of the cotyledons. The direction of the bending was such that the resulting change in position of the cotyledons would normally pull them from the testa. This bending manifested itself before the bending due to gravity and so merged with the bending induced by gravity that in the earlier series of experiments no distinction was made between it and the influence of gravity.

The experiments of Series I and II led to the conclusion that the position of the critical point does not depend upon the nutrients supplied nor the growth-promoting substances, but upon some factor which prevents cell enlargement, probably the relatively rigid condition of the cell wall or some factor (possibly an inhibiting factor) in the protoplasm of the cells of that region.

It made no appreciable difference in the degree of bending, or in the distance of the critical point from the base of the cotyledons, whether the cotyledons were intact, wholly removed or only the upper halves removed. Even when portions of the uppermost region of the hypocotyl were removed the position of the critical point did not change. The point of demarcation between the immature cells and the mature cells is surprisingly sharply defined (2.5 cm. below the cotyledons). If nutrients and growth-promoting substances are involved in the bending effect of the hypocotyl, it must be concluded from the authors' results that these substances were evenly distributed in the seedling, or had already been translocated from some obscure center of emanation as far as the critical point, so that their effect became evident within the period of time necessary to influence the given cells. Presumably the younger cells are affected by given quantities of nutrients and growth-promoting substances in less time than is required for the same quantities to exercise their influence upon older cells.

These facts point to the metabolic activity of the protoplasm in the phenomena of growth by cell enlargement. They favor the view that cells enlarge by the progressive elaboration of solutes by the protoplasm which absorb water (either directly or only after the water was imbibed by the protoplasm) by a process of osmosis, from the environment or from other cells. The hydrostatic pressure which is evoked by the influx of water would then strain the cell wall which would increase the distance between the micells. The strain need never be so great that new material which is deposited into the cell wall does not prevent the strain from going beyond the limits of elasticity. These simultaneous activities of the protoplasm cause growth by cell enlargement. This kind of growth should be regarded as a truly physiological process.

LITERATURE CITED

Anderson, B., and Kerr, T., *Ind. Eng. Chem.*, 30 (1938): 48.

Bailey, I. W., *Ind. Eng. Chem.*, 30 (1938): 40.

Beck, W. A., *Plant Physiol.*, 3 (1928): 413.

Beck, W. A., Schocken, K., and Donnelly, Sr. M. W., THESE STUDIES, 2 (1938): 107.

Boysen-Jensen, P., Translated by Avery, G. S., and Burkholder, P. R., Growth Hormones in Plants, McGraw-Hill, New York, 1936.

Buck, L., *Botan. Centr. Beihefte, A.*, 53 (1935): 340.

Dijkman, M. J., *Rec. trav. botan. néerland.*, 31 (1934): 391.

Fliry, M., *Jahrb. wiss. Botan.*, 77 (1932): 150.

Herzog, W., *Planta*, 1 (1925): 116.

Heyn, A. N. J., *Rec. trav. botan. néerland.*, 28 (1931): 113. *Protoplasma*, 19 (1933): 78.

Kögl, F., *Naturwissenschaften*, 25 (1937): 465.

Pfeffer, W., Pflanzenphysiologie: Ein Handbuch der Lehre von Stoffwechsel und Kraftwechsel in der Pflanze, Bd. II, Kraftwechsel, W. Engelmann, Leipzig, 1881, 2nd. ed., 1904.

Schwendener, S., and Krabbe, G., *Jahrb. wiss. Botan.*, 25 (1893): 85.

Thimann, K. V., and Bonner, J., *Proc. Roy. Soc.*, London, B., 113 (1933): 126.

Ursprung, A., *Plant Physiol.*, 10 (1935): 115.

Ursprung, A., and Blum, G., *Ber. deut. botan. Ges.*, 34 (1916): 525. *Biol. Zentr.*, 40 (1920): 193. *Jahrb. wiss. Botan.*, 63 (1924): 1.

Vries, H. de, *Jahrb. wiss. Botan.*, 14 (1884): 427.

Went, F. A., *Plant Physiol.*, 13 (1938): 55.

Went, F. W., and Thimann, K. V., Phytohormones, Macmillan Co., New York, 1937.

Wergin, W., *Angew. Chem.*, 49 (1936): 843.

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STIMULATION OF RAT SKIN RESPIRATION BY ULTRAVIOLET IRRADIATION IN VIVO

BY SISTER MARY MICHAELLA LANE, S.C., AND
REV. CORNELIUS H. JANSEN

FARDON, Ruddy, Loofbourow, *et al.*,[1] have demonstrated the production of a respiratory-stimulating factor by ultraviolet irradiation from yeast and animal cells *in vitro.* The present experiments were undertaken to determine if ultraviolet irradiation of animal tissue *in vivo* would also result in stimulation of respiration.

A few investigators have reported similar tests on plant cells. Masure[2] found that the irradiation of etiolated pea seedlings with ultraviolet rays of about 3650 Å wavelength temporarily increased their respiration. Wynd, Fuller and Reynolds[3] observed stimulation of respiration in tomato plants after "injurious" raying. Fardon has found that X-rays have a stimulatory effect on respiration of yeast cells[4] and of rat skin[5] *in vivo.*

Preliminary experiments in these laboratories had already been performed by Ruddy,[6] which indicated that such stimulation might be obtained by ultraviolet rays.

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[1] Fardon, J. C., Carroll, Sr. M. J., and Ruddy, Sr. M. V., THESE STUDIES, 1 (1937): 17, 41; Fardon, J. C., Norris, R. J., Loofbourow, J. R., and Ruddy, Sr. M. V., *Nature,* 139 (1937): 589.

[2] Masure, M. P., *Botan. Gaz.,* 93 (1932): 21.

[3] Wynd, F. L., Fuller, H. J., and Reynolds, E. S., *Ann. Missouri Botan. Gardens,* 22 (1935): 837.

[4] Fardon, J. C., unpublished results.

[5] Fardon, J. C., Sullivan, W. A., Brotzge, G., Loeffler, Sr. M. K., and Andrus, Sr. M. B., THIS ISSUE, p. 203.

[6] Ruddy, Sr. M. V., unpublished results.

EXPERIMENTAL

In the present experiments 19 female albino rats, one year old, were used. One rat was used in each experiment. The abdomen of the rat was shaved free of hair and covered with a canvass cloth in which was cut a hole 3.5 cm. square, through which a like portion of skin of the abdomen was exposed to the irradiating rays. This area was carefully outlined on the skin before removal of the cloth.

In each experiment the animal was irradiated on this exposed area by means of a Sperti quartz mercury arc, of 110-volt and 75-watt capacity, at a distance of 25 cm. and for a period of 15 minutes.

After intervals, varying from 0 to 4 hours after irradiation, this area of skin, as well as a similar unexposed area of the abdomen, was carefully excised and immersed in Ringer's phosphate-glucose solution, kept at body temperature. Each piece of skin was stripped of all fatty and connective tissue and cut into 2 mm. squares.

Eight manometer flasks were each charged with 3.1 cc. Ringer solution (pH 7.3) in the outer flask and 0.2 cc. KOH in the inner well. Into 3 flasks irradiated skin fragments were placed, and into 3, non-irradiated or control skin fragments, while 2 manometers were used as barometric controls. The amount of skin in each flask averaged 35 mg. of dry weight.

The respiration period was 2 hours, with readings taken every hour. At the conclusion of the respiration period, each portion of skin fragment was washed in a Gooch crucible and dried in the oven for 20 hours at 110° C. The Q_{O_2} (cu. mm. O_2/mg./hr.) was calculated from the weight of the dry tissue. The time interval between the beginning of the excision to the beginning of the respiration period averaged 30 minutes.

TABLE I

Comparison of Average Respiration Quotients of Ultraviolet Irradiated and Non-Irradiated Rat Skin

Number of Experiments	Interval between Irradiation and Excision in Hours	Average Q_{O_2} Control	Average Q_{O_2} Experimental	Per Cent Stimulation
3	0	.539	.635	18
3	1	.428	.469	10
7	2	.480	.525	9
2	3	.473	.532	12
4	4	.544	.596	10

The results of the experiments have been tabulated in Table I. Because of the variations usually encountered in all biological measurements it was deemed best to average the results as much as possible. Consequently the average Q_{O_2} of all the experimental flasks of experiments, in which the time between irradiation and excision was the same, was compared with the average Q_{O_2} of the corresponding control flasks. A few flasks in which the Ringer solution had been contaminated with the KOH of the inner well were omitted from the tabulation. The variations in results for individual experiments ranged from −15 per cent stimulation to +62 per cent, and out of the 19 experiments, 4 gave negative stimulation (−15, −4, −9 and −2 per cent) and 15 experiments gave positive.

As can be seen from Table I, there was a definite stimulation of respiration of the rat skin by ultraviolet irradiation *in vivo*. This stimulation persisted without apparent diminution for at least 4 hours. The effect may be attributed to some direct influence of the ultraviolet rays upon the cells of the skin.

In vitro experiments[1] have given evidence that the stimulatory effect is caused by certain substances secreted by cells when placed under the abnormal environment of irradiation; it seems logical that these same factors operated *in vivo* to cause the increase in stimulation.

The authors feel that the stimulation should not be attributed to the effects of erythema or consequent infiltration of luecocytes, for if these were the principal cause of the stimulation of respiration, the stimulation would have been more gradual and risen to a climax some 2 or 4 hours after irradiation.

SUMMARY

Rat skin irradiated *in vivo* by ultraviolet light is stimulated in its respiration, and this stimulation persists for at least 4 hours after irradiation. It appears to be due to a direct effect of the radiations on the skin and not to the consequent erythema.

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